

A Novel NIR Spectroscopy Correlation Approach to Amino Acid Analysis of Soybean Proteins for Composition Improvements

V. I. Prisecaru

and

I.C. Baianu

FSHN Department – College of ACES

AFC-NMR & NIR Microspectroscopy Facility

University of Illinois at Urbana-Champaign

Urbana, Illinois USA



1. Introduction

- Overall Goals**
- Research Objectives**
- Background**
- Referenced Summary of Relevant Work**

Table of Contents

- **Project Summary**
- **Project Description**
 - 1. Introduction**
 - Overall Goals
 - Research Objectives
 - Previous Studies: Referenced Summary of Previous Work
 - Preliminary Data
 - 1. Rationale and Significance**
 - 2. Research Methods**
 - Proposed Studies and Techniques
 - Application of Results, Future
 - Limitations and Advantages of Methodologies
 - 1. References**
 - 2. Appendices**

PROJECT SUMMARY

In the following slides a research project will be presented in which state-of-the-art FT-NIR instruments were evaluated and utilized, data analysis and calibration methodology were substantially improved. The goal was to allow rapid NIR analysis of the large number of soybean samples required for improving the genetics of soybean seed composition for agricultural cost savings and human health food applications.

PROJECT SUMMARY, cont'd

Calibrations are developed for three selected amino acid groups that include essential amino acids for identified soybean accessions.

Conventional “wet chemistry” analytical methods are time-consuming and costly. As a result, soybean breeders and researchers have an imperative need to utilize faster and less expensive methods. NIR Spectroscopy is a rapid and inexpensive method for composition analysis for academia and industry. Recent advancements in instrumentation design, such as the application of the Diode Array (DA) technique and the Fourier Transform (FT) IR and NIR techniques, have significantly improved overall instrument performance and advancement in the field of grain analysis.

Project Description

Overall Goals

- To design improved approaches to NIR calibrations for amino acid composition analysis of soybean proteins, soybeans and soybean food and feed matrix formulations
- To develop NIR calibration transfer methodology between different laboratories for various food and feed applications

Research Objectives

To produce NIR amino acid calibrations for a small group of three amino acids.

To investigate and develop methodology for NIR spectra pre-processing and data analysis to improve both the accuracy and reliability of NIR measurements of soybean seed composition.

To develop and optimize NIR Spectroscopy calibrations for determination of the above-mentioned amino acids in soybean seeds.

Research Objectives

To obtain reproducibly calibration plots of the above-mentioned four individual amino acids in soybean lines, using PLS-1 and PLS-2 regression algorithms

To perform multivariate analysis to better resolve the individual amino acid calibrations.

To carry out the analysis and comparison of soy proteins of similar amino acid composition in powder vs. gel vs. liquid suspension, in order to investigate the matrix effect on the NIR calibrations.

To compare reduced and unreduced soybean protein calibrations, to improve the cysteine vs. cystine NIR calibrations.

Research Objectives

- 1. To compare our results with the results of other laboratories' data, in order to investigate the transferability of NIR calibrations among different laboratories.**
- 2. To compare results with ^{13}C (Waltz;WAHUHA)) NMR and GC-MS primary data for amino acid composition.**

Research Objectives

1. To compare our results with the results of other laboratories' data, in order to investigate the transferability of NIR calibrations among different laboratories.
2. To compare results with ^{13}C (Waltz;WAHUHA)) NMR and GC-MS primary data for amino acid composition.

Objectives, cont'd

Evaluate multivariate analysis methodology for NIR calibrations in order to determine soybean protein and soybean amino acid residue contents (go to wiki, or google, etc., and learn some)

Investigate the potential of NIR spectroscopy for developing calibrations for amino acids and amino acid mixtures [amino acid triple matrix method] [specify which amino acids and which mixtures – specify aa's, but for the mixtures, refer to the table in the next page]

Generate NIR calibrations of the soybean protein amino acid residues specified in the table on the next page, based on high-resolution nuclear magnetic resonance analysis

Background

The soybean:

- more than 3 Billion bushels produced in the US each year (USDA, 2007)
- Major source of plant protein and oil (and a high-level plant source of Methionine and Tryptophan)
- Protein content from different soybean cultivars vary greatly
 - Some have over 50% protein (dry wt.)
 - Some accessions show significantly higher Methionine (~19%*) and Cysteine levels

*Kuiken, 1948

Project Description

Overall Goals

- To design improved approaches to NIR calibrations for amino acid composition analysis of soybean proteins, soybeans and soybean food and feed matrix formulations
- To develop NIR calibration transfer methodology between different laboratories for various food and feed applications

Research Objectives

Research Objectives

- 1. To compare our results with the results of other laboratories' data, in order to investigate the transferability of NIR calibrations among different laboratories.**
- 2. To compare results with ^{13}C (Waltz;WAHUHA)) NMR and GC-MS primary data for amino acid composition.**

Research Objectives

Objectives that need to be addressed in order to realize NIR's utility in practical food applications and lab analysis:

Generate an NIRS calibration for all of the amino acid residues in soybean proteins that are determined by a primary method

Obtain an NIRS calibration for selected groups of three amino acid triplets, such as **Arg-Lys-Glx**, **Asx-Ala(or Val)-Pro**, or **(Met+Cys)-Arg-Val**.

- (Can such determinations result in significant savings of time and money in research and food industry labs?)

Obtain appropriate and accurate values for use in precision formulation matrices.

Research Objectives

To produce NIR amino acid calibrations for a small group of three amino acids.

To investigate and develop methodology for NIR spectra pre-processing and data analysis to improve both the accuracy and reliability of NIR measurements of soybean seed composition.

To develop and optimize NIR Spectroscopy calibrations for determination of the above-mentioned amino acids in soybean seeds.

Research Objectives

To obtain reproducibly calibration plots of the above-mentioned four individual amino acids in soybean lines, using PLS-1 and PLS-2 regression algorithms

To perform multivariate analysis to better resolve the individual amino acid calibrations.

To carry out the analysis and comparison of soy proteins of similar amino acid composition in powder vs. gel vs. liquid suspension, in order to investigate the matrix effect on the NIR calibrations.

To compare reduced and unreduced soybean protein calibrations, to improve the cysteine vs. cystine NIR calibrations.

Background

Soybean Uses

Main Soybean Growing Countries:

- United States, Brazil, Argentina, China and India

Some final products from soybean processing:

- Foods, Nutraceuticals, soy isoflavones
- TVP
- Animal feed
- Adhesives, Fibers, Lining
- Foams
- Fertilizers

Usage in Industry

- Developmental Labs and Grain Labs in industry have been reluctant to use NIR because of the low quality of instruments available (until recently) and to an extent, the lack of proper calibrations
- It has already been used in the area of new grain development, genetic selection and cross-breeding.
- Because of its high sensitivity, NIR is useful as a rapid and inexpensive screening tool, despite not having very high resolution, if a robust and accurate calibration can be generated.

Current Status

- The Food Industry and Nutritional Sciences have a great need for rapid techniques that are economical, accurate, reproducible and nondestructive.
- Protein Quality is an important processing and nutritional attribute

Soybean protein and amino acids

– best method

- The methods that exist for judging protein quality are mostly destructive, and possess severe limitations, like changing the structure of amino acids before they're quantitated.
- SS NMR is an established method used to identify the aa residues, but has resolution limitations. However, NMR can be done in liquids or gels that improves the resolution.
- Drawback: NMR takes much longer than NIR
- NIR is a powerful *secondary* technique

UIUC NIR Soybean Database

- Our high-resolution NIR calibrations and methodologies were employed to carry out a large number of protein and oil composition analyses of soybean seeds (:80,000) for breeding and selection purposes, over a period of three years.
- A wide variety of soybean developmental lines and more than 5,000 exotic soybean germplasm accessions were thus characterized accurately and reproducibly (Source: UIUC Soybean NIR Database).
- Such results demonstrate the usefulness of this novel NIR approach for soybean selection and breeding purposes. They also validate our NIR calibrations undertaken in parallel with the higher resolution (but slower and more expensive) NMR measurements.

Applications

- R & D
 - Food Formulations and Protein Quality / Amino Acid Composition Analysis
- Food Developments
- Food Safety and Microbiology Applications
- Health Foods
- Nutraceuticals
- Nutrition Research
 - Agricultural Feeds and Pet Foods

Biomedical Applications

High-resolution NIR Chemical Imaging may also enable rapid and sensitive analyses with micro-arrays for Nucleic Acids, multiple Molecular Bioassays, Automated Proteomics, Biotechnology, Biomedical & Pharmaceutical Applications, such as those aimed at early Detection of Cancer and Prevention.

References to Previous Work

- V. Prisecaru Publications
- I.C.Baianu et al. Publications
- Orf et al. Publications, etc.

VIP References

- Baianu, I.C., and Prisecaru, V.I. 2005. Complex Systems Analysis of Cell Cycling Models in Carcinogenesis. Preprint arXiv/q-bio.OT/0406045. CERN/CDS online. 23 pages.
- Gonzalez de Mejia, E., Vásconez, M., Prisecaru, V.I. and Nelson, R. Measuring Lectin Concentration in Selected Soybean Genotypes, 2006, Article in Submission.
- Baianu, I.C., Lozano, P.R., Prisecaru, V.I. and Lin, H.C., 2004. Novel Techniques and Their Applications to Agricultural Biotechnology, Health Foods and Medical Biotechnology. Applied Biotechnology, Food Science and Policy. In press.
- Baianu, I.C., You, T., Costescu, D.M., Lozano, P.R., Prisecaru, V.I. and Nelson, R.L. 2003. High Resolution Nuclear Magnetic Resonance and Near Infrared Determination of Soybean Oil, Protein and Amino Acid Residues in Soybean Seeds. 94th AOCS Symposium Proceeding. In press.
- De Mejia, E.G., and Prisecaru, V.I., 2003. Lectins as bioactive plant proteins: new frontiers in cancer treatment. Critical Reviews in Food Science and Nutrition.
- Painter, J.E., Lee, Y.K., Prisecaru, V.I., and Hieggelke, J.B., 2003. Consumer preference for energy bars based on macronutrient composition, gender, consumption frequency and participation in weight training. (Article in submission).
- Painter, J.E., and Prisecaru, V.I., 2002 June. The effects of various protein and carbohydrate ingredients in energy bars on blood glucose levels in humans. Cereal Foods World, 47(6):236-241.
- Painter, J.E., and Prisecaru, V.I., 2003. Assessing vitamin C and E deficiencies in U.S. adults and suggesting ways to reduce deficiencies. Nutrition Today.

VIP References, cont'd

- Shay, N.F., Ezzatkhah, M., Prisecaru, V.I., and Mangian, H.F., 1999. Zinc deficiency does not affect self-selection of dietary protein in 2-choice macronutrient preference tests using albumin or purified amino acids. *FASEB J.* 13(5):197-6.
- Assessing vitamin C and E inadequacies in U.S. adults and suggesting ways to reduce inadequacies. ADA Conference, St. Louis, MO 2001.
- The effects of various macronutrient ingredients in energy bars on blood glucose levels. ADA Conference, St. Louis, MO 2001.
- The effects of various macronutrient ingredients in snack bars on energy levels, satiety and hunger. FFH Conference, Urbana, IL, 2002. Abstract.
- The starting point for a healthy diet, ADA 2003 Oct. Abstract.
- Poster presentation: Assessing vitamin C and E deficiencies in U.S. adults using NHANES III and the 2000 DRIs. ADA Conference, St. Louis, MO 2001
- Poster presentation: The effects of various protein and carbohydrate ingredients in snack bars on blood glucose levels. ADA Conference, St. Louis, MO 2001.
- Poster presentation: Assessing vitamin C and E deficiencies in U.S. adults and suggesting ways to reduce deficiencies. IDA meeting Chicago, 2002
- Poster presentation: The effects of various protein and carbohydrate ingredients in laboratory-made energy bars on blood glucose levels. IDA meeting Chicago, 2002.
- Poster presentation: The effects of various protein and carbohydrate ingredients in snack bars on energy levels, satiety and hunger. FFH Conference, Urbana, IL, 2002.
- Poster presentation: The starting point for a healthy diet. ADA 2003 Oct.
- Abstract: Painter, J.E., North, J., and Prisecaru, V.I., 2003. The starting point for a healthy diet: where popular diets and the governmental authorities agree. (2003 – JADA).

References by I.C. Baianu et al

- Baianu, I.C. 1993. Solid-State NMR Analysis of Soybeans and Foods. *Value Added Soybean Summit Meeting*, Washington, DC.
- Baianu, I.C. and T. F. Kumosinski. 1992. *Physical Chemistry of Food Processes*. 2nd edn. Vol. **1**, Van Nostrand-Reinhold: New York, NY.
- Baianu, I.C., Pessen, H., Kumosinski, T.F., Editors. 1994. *Physical Chemistry of Food Processes: Principles, Techniques and Applications*, Vol.**2**, Chapman and Hall: London, New York and Melbourne.
- Baianu, I.C. 1994. Thermodynamic linkage of specific ion binding and hydration properties of soy glycinins and conglycinins determined by NMR techniques. *Biophys.J.*, **66**: 246.
- Baianu, I.C., Tiefeng You, Jun Guo, and Randall L. Nelson. 2002. Calibration of Dual Diode Array and Fourier Transform NIR Spectrometers for Composition Analysis of Single Soybean Seeds in Genetic Selection, Cross-Breeding Experiments., *Proceedings of the 9th Biennial Conference of the Cellular and Molecular Biology of the Soybean*, August 11-14, p.508. (Soy2002 Platform presentation).
- Baianu, I.C.T, You, D.M. Costescu, V. Prisecaru, P. Lozano and R.L. Nelson. 2004a. High-resolution NMR and Near Infrared Determination of Soybean Oil, Protein and Amino Acid Residues in Soybean Seeds. In "*Analysis of Oil Seeds*". D. Luthria, Ed., Champaign: AOCS Publs., pp.193-240
- Baianu, I.C., D.M. Costescu, T. You, V., P. Lozano, N.E. Hofmann and S.S. Korban. 2004b. Near Infrared Microspectroscopy, Fluorescence Microspectroscopy, Infrared Chemical Imaging and High-resolution NMR Analysis of Soybean Seeds, Somatic Embryos and Single Cancer Cells. In "*Analysis of Oil Seeds*". D. Luthria, Ed., Champaign: AOCS Publs., pp 241-273.
- Baianu, I.C., Lozano, P.R., Prisecaru, V., and Lin, H.C. 2008. Applications of Novel Techniques to Health Foods, Medical and Agricultural Biotechnology. *Quant. Biol. arXiv*, 2008-Update.

References by I.C. Baianu et al, continued:

Guo, J. and Ion C. Baianu. 2002. Determination of Soy and other Health Foods Composition by Fourier Transform Near Infrared Reflectance Spectroscopy, in *Proceedings of the 9th Biennial Conference of the Cellular and Molecular Biology of the Soybean*, p.506.

Guo, J. and Ion C. Baianu. 2002. Rapid Determinations of Soybean Isoflavones, Soy and Health Foods Composition by Fourier Transform Near Infrared Reflectance Spectroscopy, *Proceedings of the China and International Soy Conference and Exhibition 2002 (CISCE 2002)*, November 6-9, pp. 391-392.

You, T., Jun Guo, Ion C. Baianu, and Randall L. Nelson. 2002. Rapid Determination of Protein, Oil, Moisture, and Isoflavone Contents of Single Soybean Seeds by Fourier Transform Near Infrared Reflectance Spectroscopy, *Proceedings of the China and International Soy Conference and Exhibition (CISCE 2002)*, November 6-9, pp. 414--415.

You, T., Jun Guo, Ion C. Baianu, and Randall L. Nelson 2002. "Determination of Isoflavone Contents in Bulk for Selected Soybean Lines by Fourier Transform Near Infrared Reflectance Spectroscopy", in *Proceedings of the 9th Biennial Conference of the Cellular and Molecular Biology of the Soybean*, pp.503-5-04.

Other References

- Orf et al. refs
 - Panthee, D.R., V.R. Pantalone, C.E. Sams, A.M. Saxton, D.R. West, J.H. Orf and A.S. Killam. 2005. Quantative trait loci controlling sulfur containing amino acids, methionine and cysteine, in soybean seeds. *Theor. Appl. Genet* 122(5): 161-6.
 - Helms, T., J. Orf, G. Vallad, and P. McClean. 1997. Genetic variance, coefficient of parentage, and genetic distance of six soybean populations. *Theor. Appl. Genet.* 94: 20-26.
 - Helms, T.C., and J.H. Orf. 1998. Protein, oil, and yield of soybean lines selected for increased protein. *Crop Sci.* 38:707-711.
- Other refs

Previous Studies

Brazil, 2003 to 2006

- Collected soybean samples in several different states in Brazil
- Analyzed amino acids in soybeans using:
 - HPLC
 - Derivatized HPLC
- Individual amino acid compositions of the soybean samples showed significantly different amino acid mean levels ($p < 0.01$ and $p < 0.02$) – **Met, Lys, and Thr**
- Tentative Conclusion: it is possible to calculate the a.a. content of a sample for several amino acids by comparison with a primary method; **however, primary data was *not* available for several essential amino acids.**

Rationale and Significance

Both major advancements in instrumentation, and improved data analysis/novel calibration methodologies are necessary to improve the accuracy and reliability of NIR for measuring low-level components such as individual amino acids.

Rationale and Significance

- High-protein, high-yield cultivars increase the soybean crop value. Conventional, or “wet chemistry”, methods are time-consuming, expensive and impractical for repetitive measurements required for genetic selection and breeding experiments to increase both protein content and the agronomic yield values of soybean cultivars
- **Faster and less expensive methods for protein, oil, moisture and amino acid analysis of soybeans are needed**

Rationale and Significance

Novel NIR instrumentation techniques – combined with improved data analysis and calibration methodologies – are essential for selecting soybean cultivars with both high quality protein composition and high agronomic yield.

Such improved NIR analysis can also result in enormous cost and time savings for the amino acid composition analysis that is required for example by soybean breeders in genetic selection experiments.

NIR AA Calibration Data

Flow-Chart of the Steps in a Novel Approach for Amino Acid NIRS Analysis of Soybeans and Proteins

Selection of Standard Samples with the widest Range of Equally-Spaced Values (ES-ROV)

Primary Methods of Analysis for:

(AA , P , M and O (oil))

*Correlation test with
Protein % D*

Precision Formulation Matrix for Calculating
Concentration Steps

Selection Criteria:

High ROV > 20%
and Low Correlation: < 90%

NIRS Measurements

Next Group of
Amino Acids

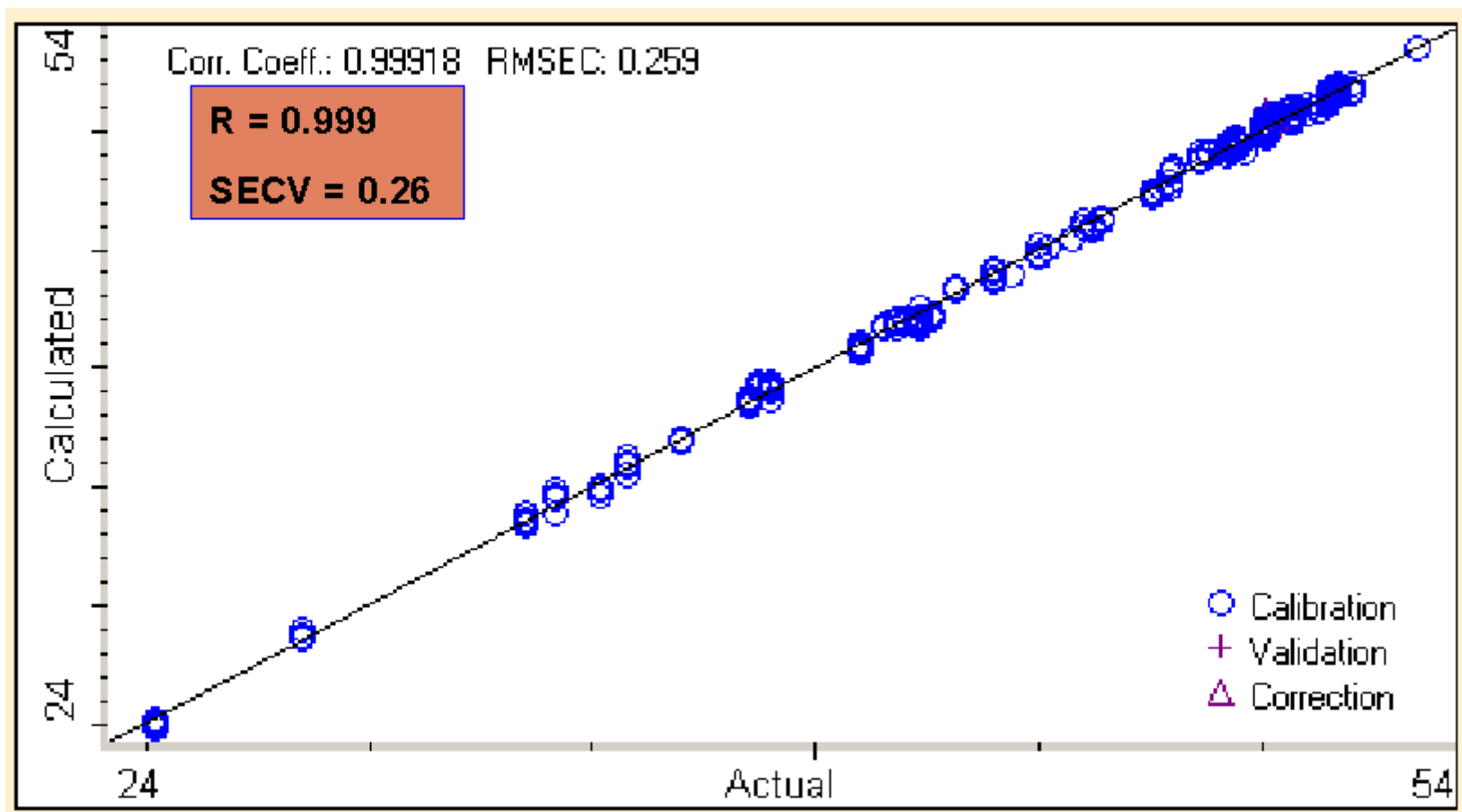
PLS-1

PLS-2 (n<6: 3 AA+P+M+O)

*Three AA Composition
Results*

*Selection of an Amino
Acid Triplet Group
for Calibration*

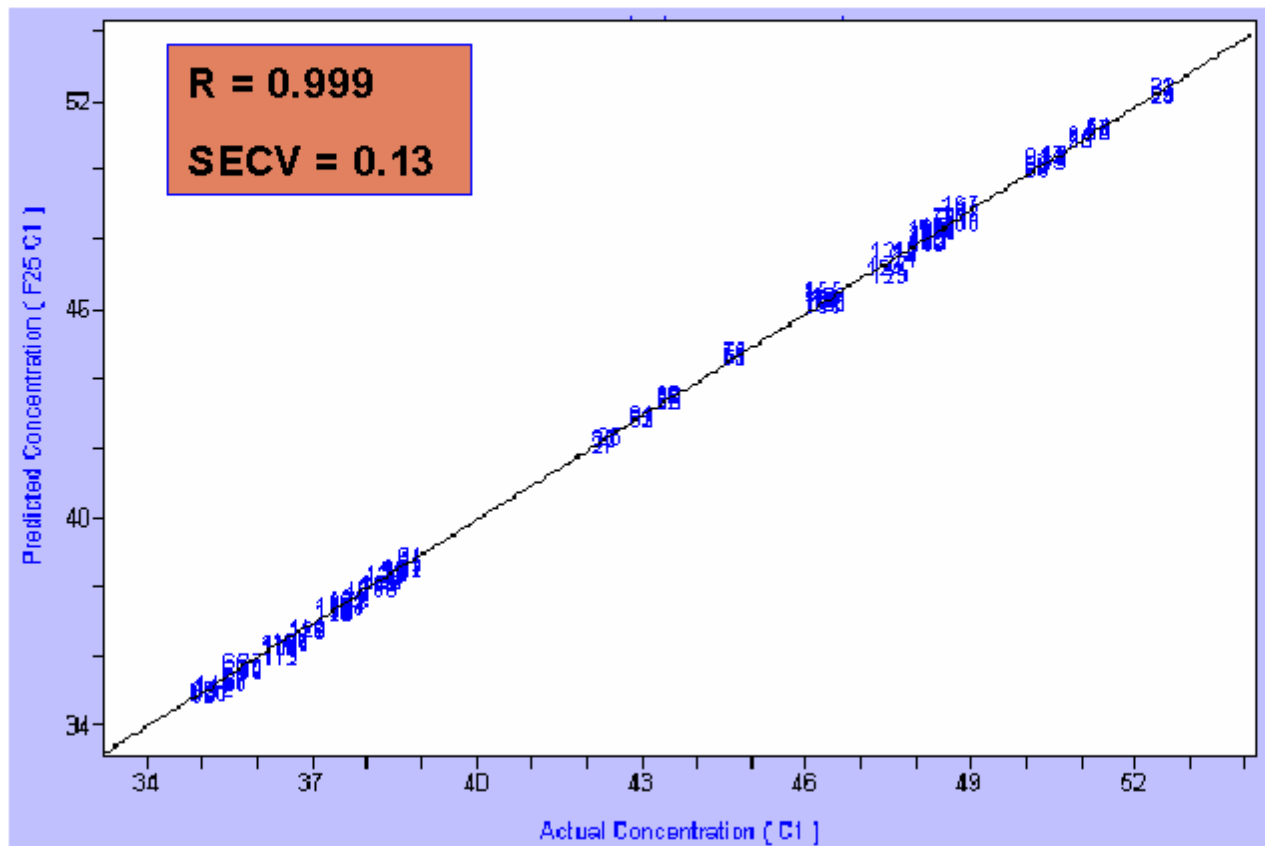
Protein Calibration for Bulk Soybean Analysis on the Spectrum One NTS FT-NIR Instrument



65 calibration standards, 20 grams for each standard, 8.9mm NIR beam size

Source: Soybean NIR Database, UIUC

Protein Calibration for Bulk Soybean Analysis on the DA-7000 Diode Array NIR Instrument



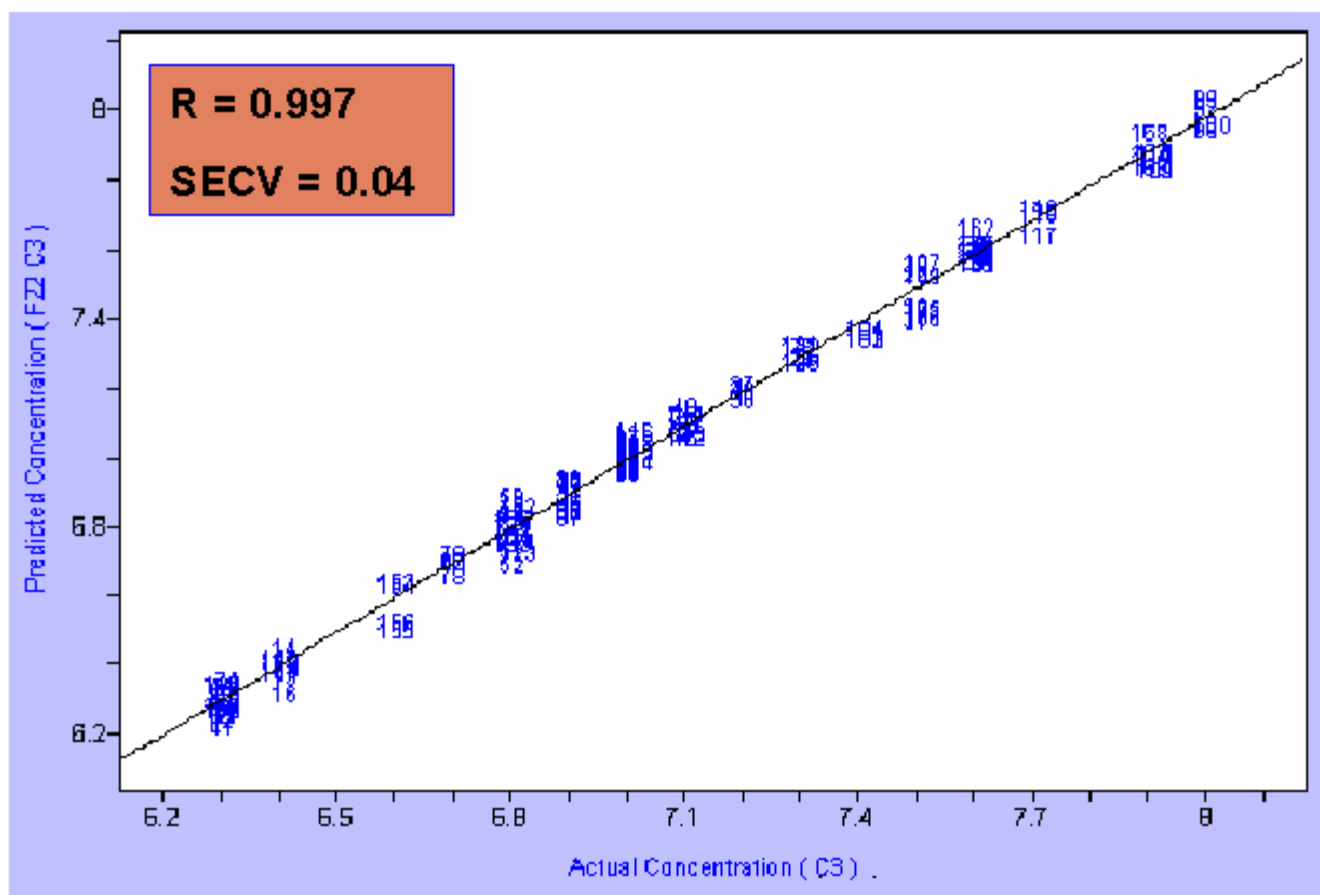
Source: Soybean NIR Database, UIUC

Calibration Results for Protein and Oil Analysis with the Perten DA-7000, Dual Diode-Array DA-NIR Instrument

Components	Protein		Oil	
	Bulk Sample	Single Seeds	Bulk Sample	Single Seeds
SECV	0.1	1.1	0.1	0.5
R	99.9%	98.5%	99.9%	98.5%

Source: Soybean NIR Database, UIUC

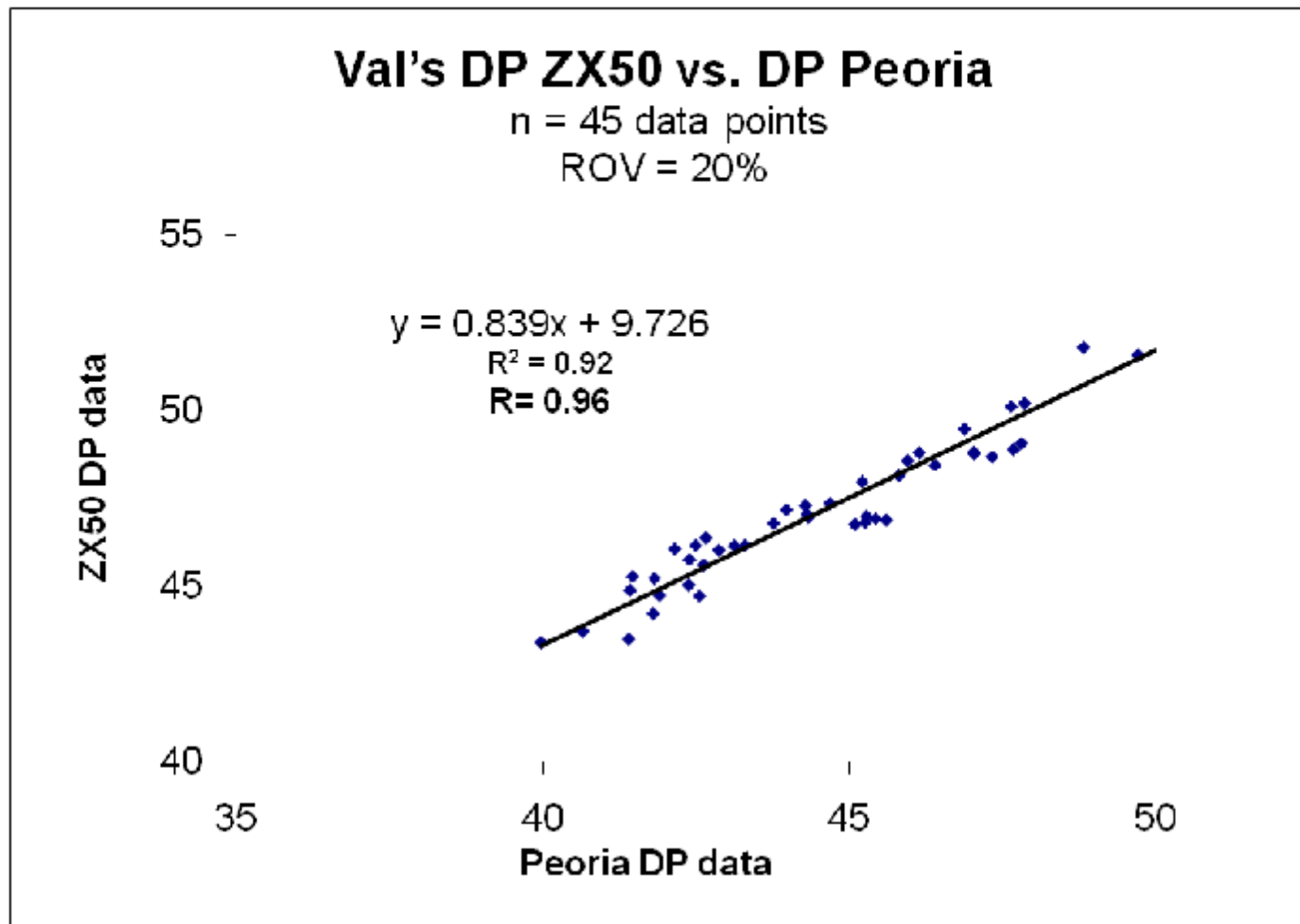
Moisture Calibration for Bulk Soybean Analysis on the DA-7000 Diode Array NIR Instrument



Source: Soybean NIR Database, UIUC

Comparison of NIR Dry Soy Protein

Data(Primary data: Sigma Method–Lowry modified)



Correlations of Amino Acids with Crude Protein in Soybeans

A.A.	R ²	R
ASX	0.634	0.796
THR	0.005	0.071
SER	0.578	0.760
GLX	0.690	0.831
PRO	0.480	0.693
GLY	0.628	0.792
ALA	0.619	0.787
VAL	0.640	0.800
ILE	0.631	0.794

A.A.	R ²	R
LEU	0.644	0.802
TYR	0.536	0.732
PHE	0.474	0.689
HIS	0.499	0.706
LYS	0.652	0.807
ARG	0.687	0.829
MET	0.548	0.740
CYS	0.498	0.706
M+C	0.538	0.733

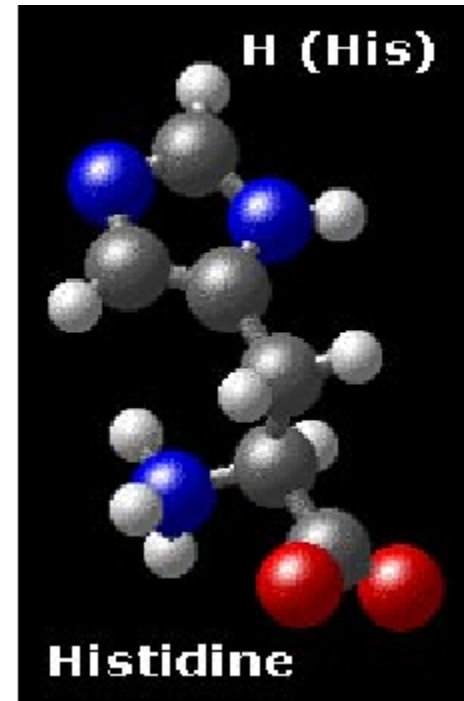
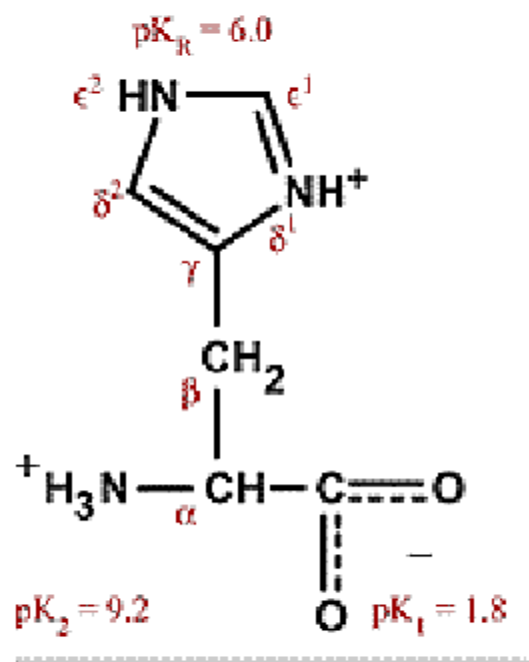
Amino Acids **Highly-Correlated** with the Dry Protein Content:

- Histidine: $R = 0.93$
- Arginine: $R = 0.90$
- Glx: $R = 0.88$
- Valine: $R = 0.87$
- Leucine: $R = 0.85 \rightarrow \textit{borderline}$

=====

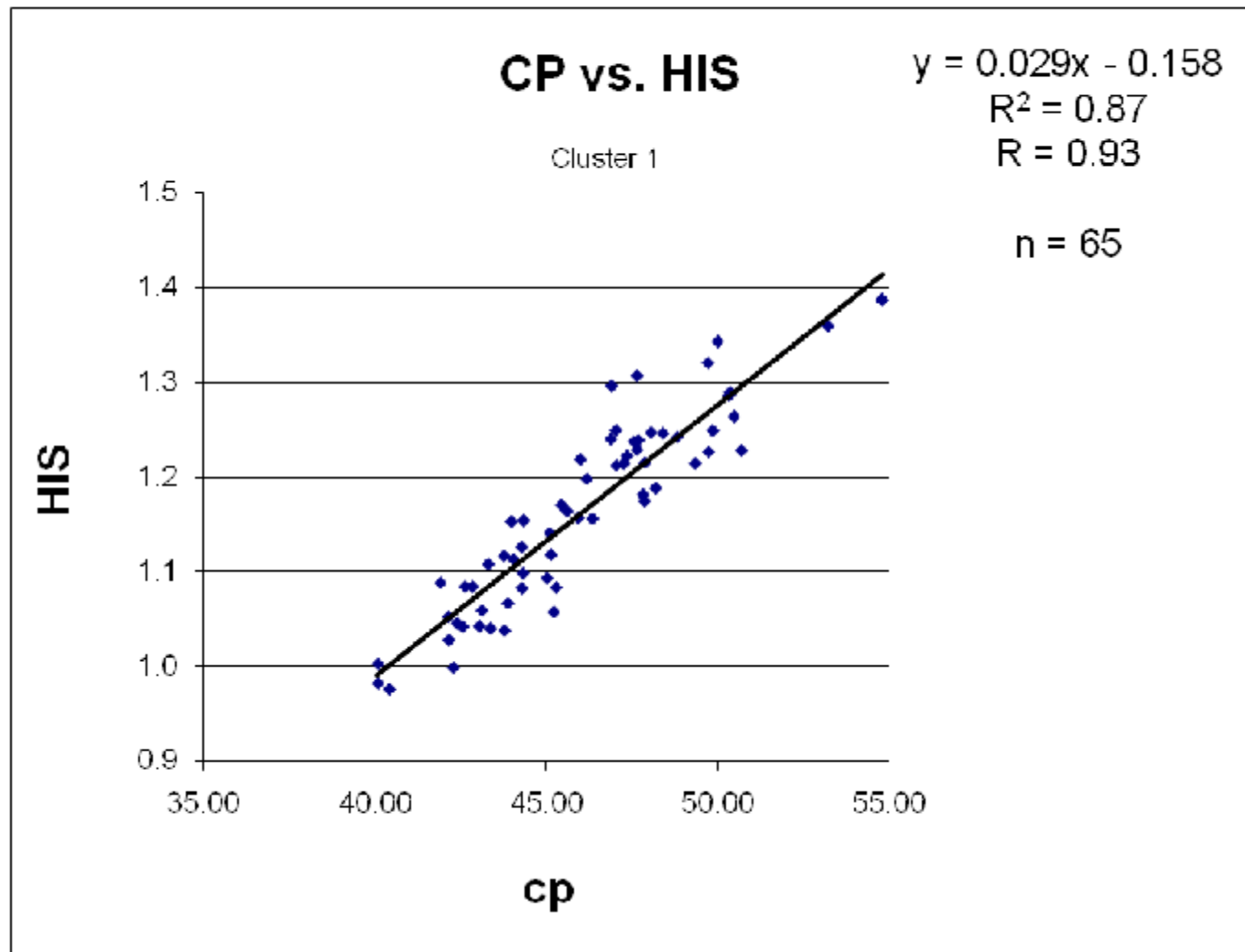
and the imino acid Proline: $R = 0.87$

Histidine

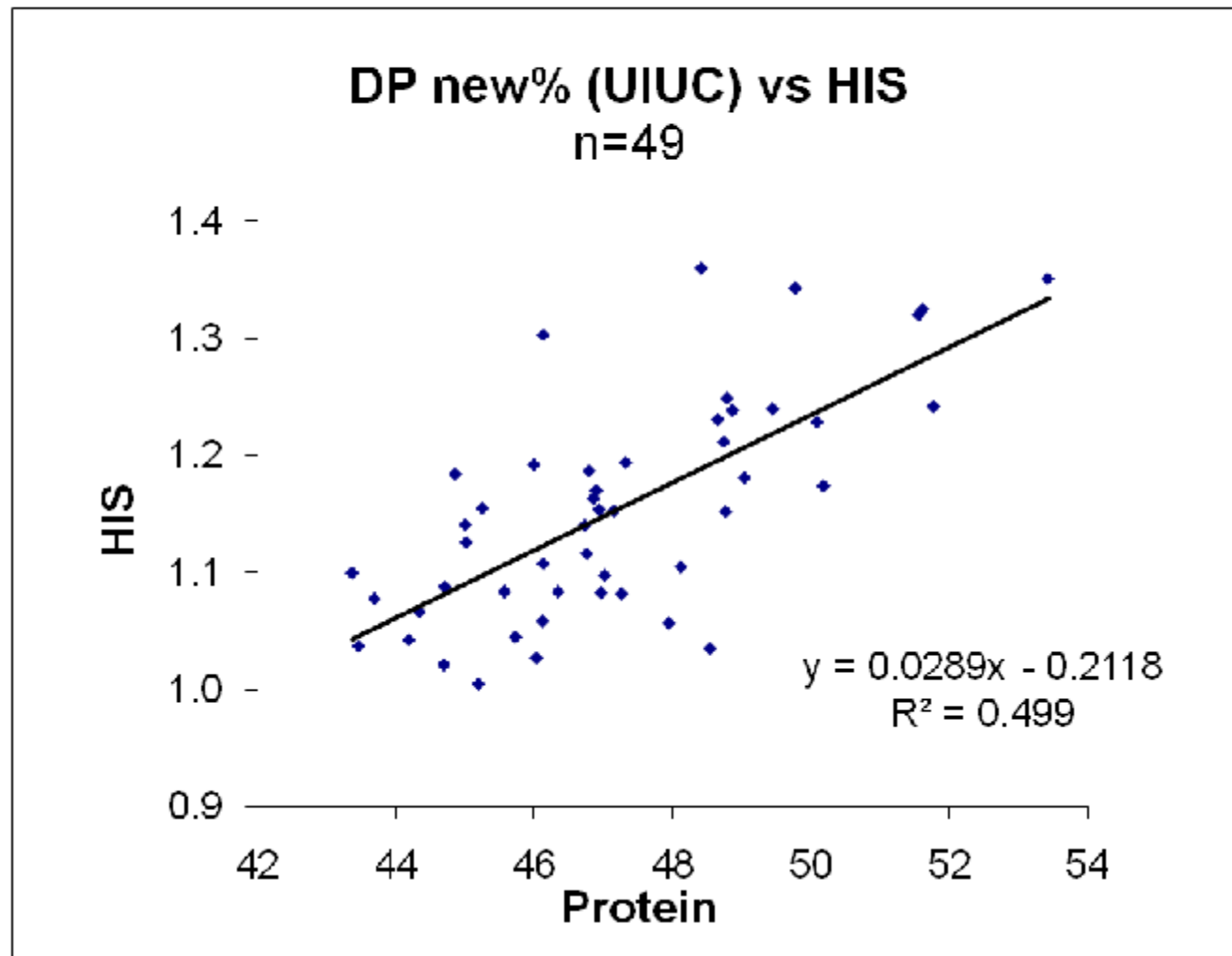


- Basic
- Polar
- Positively charged
- $pK_1 = 1.8$
- $pK_2 = 9.2$
- $pK_R = 6.0$ (NH⁺)

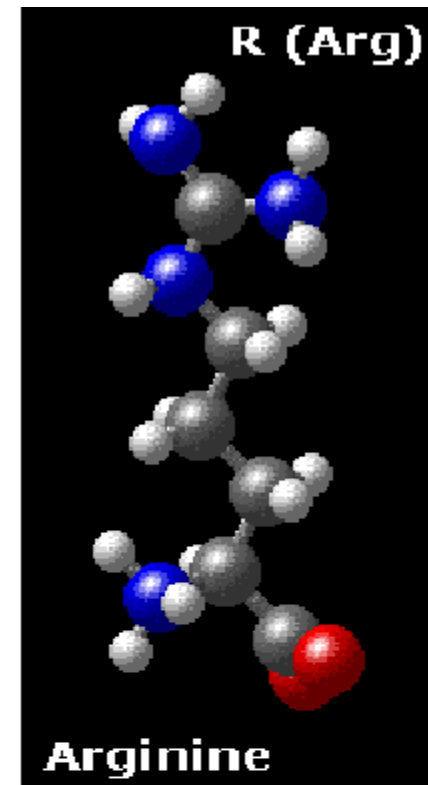
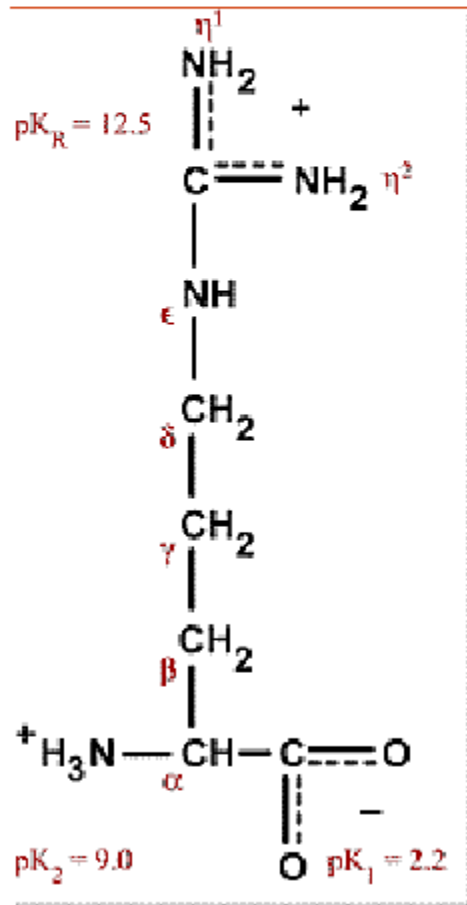
Histidine: His vs. % Dry Protein



Histidine: His vs. % Dry Protein

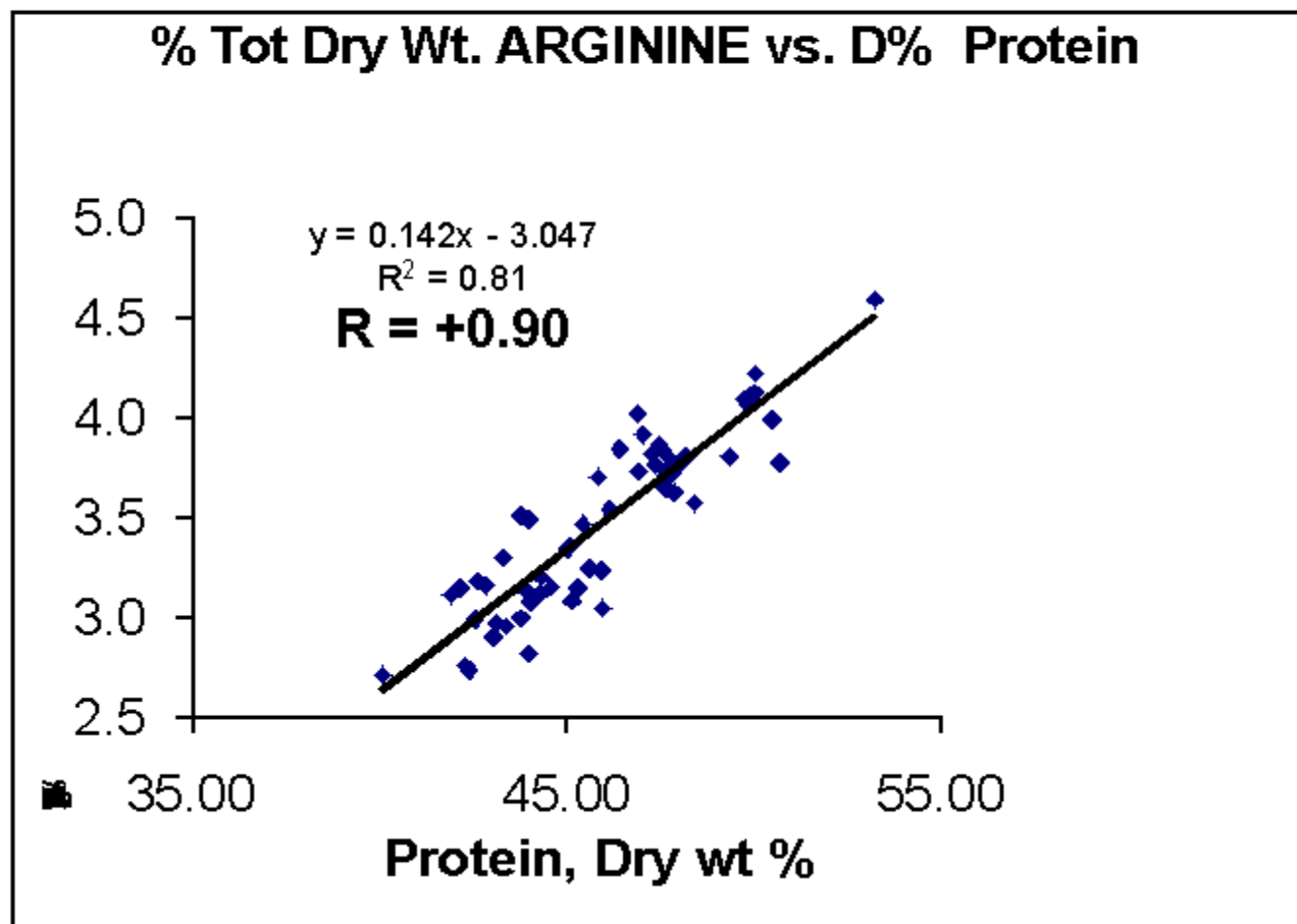


Arginine

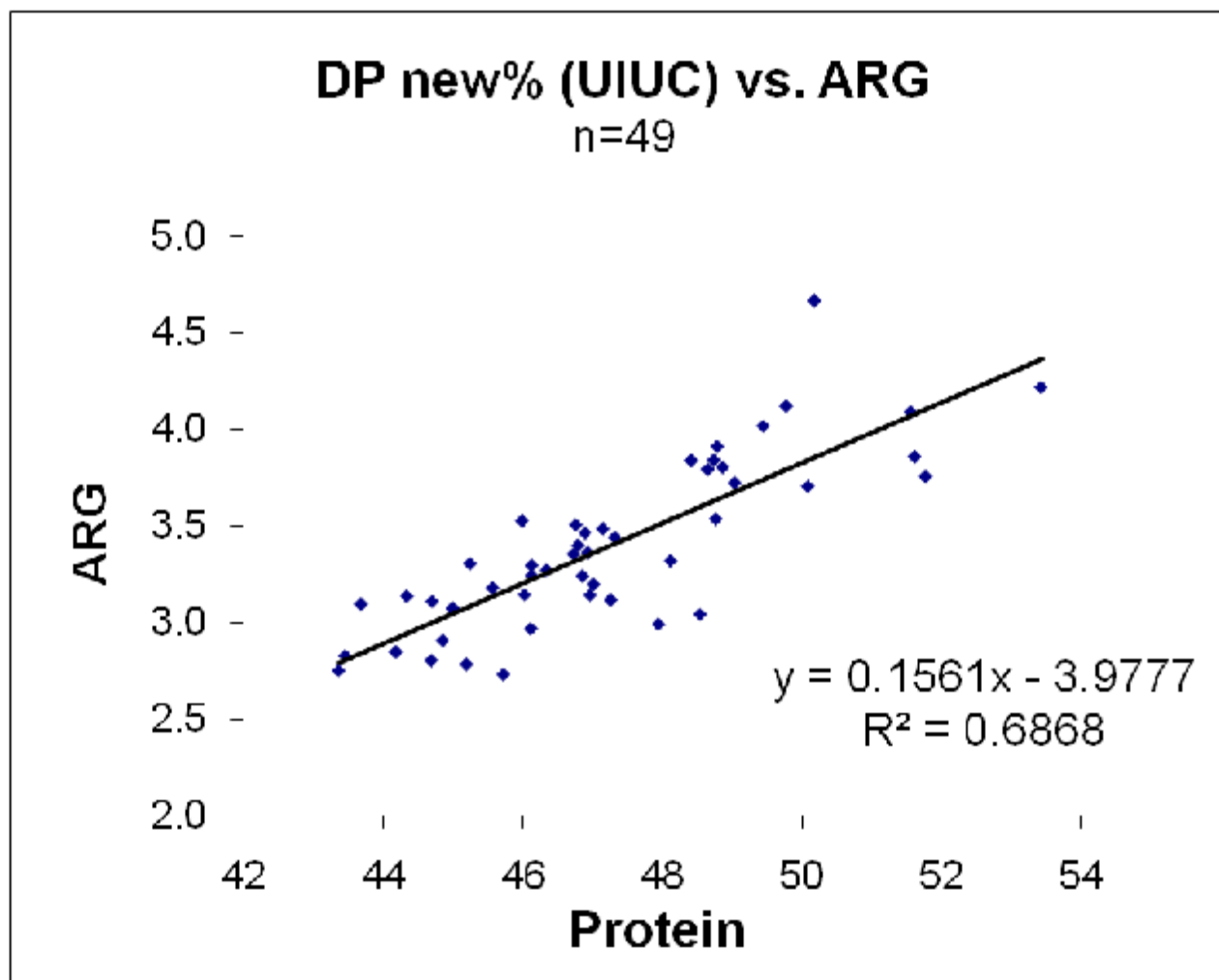


- Polar
- Positively charged
- $pK_1 = 2.2$
- $pK_2 = 9.0$
- $pK_R = 12.5$

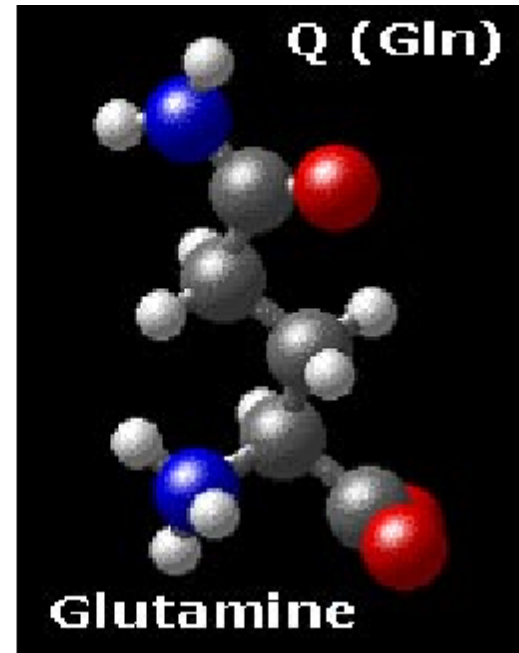
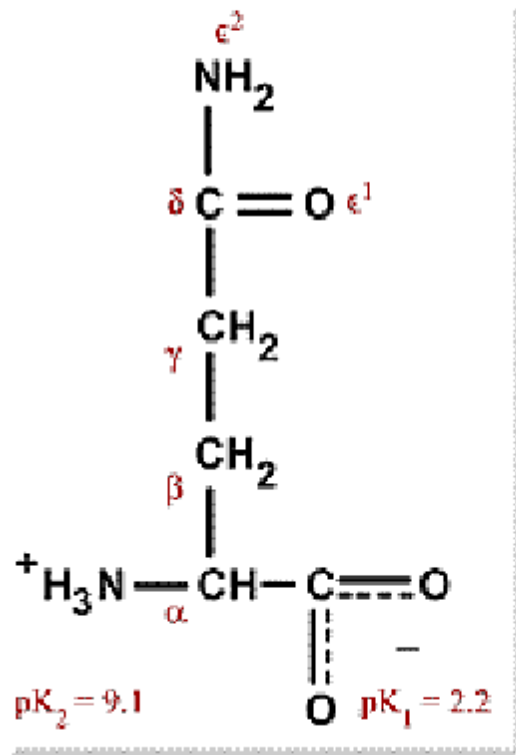
Amino Acid Contents of Soybeans Determined by GCMS and Correlation with Total Dry Protein



Amino Acid Content of Soybeans Determined by GCMS: Correlation with Total Dry Protein

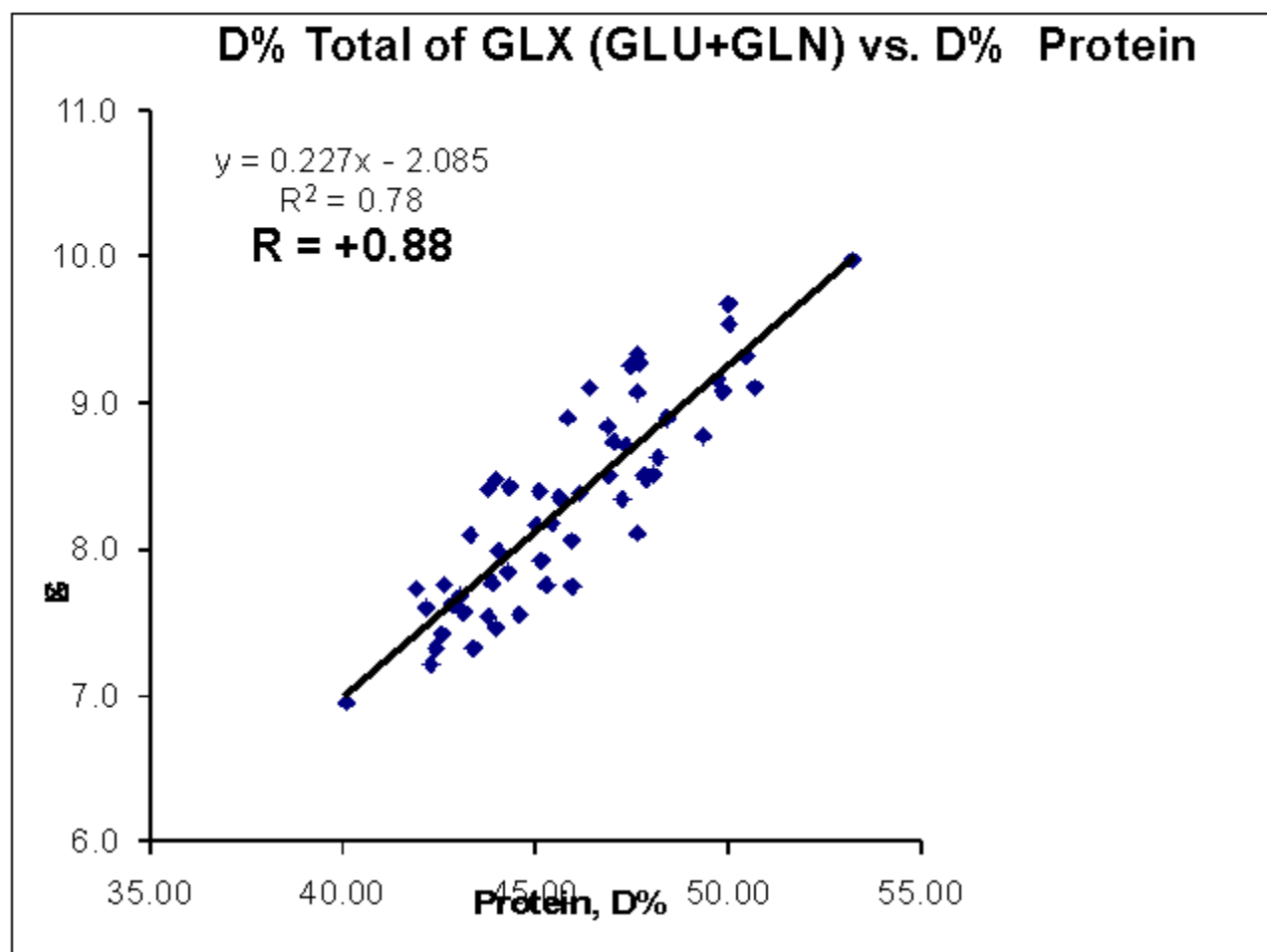


Glutamine

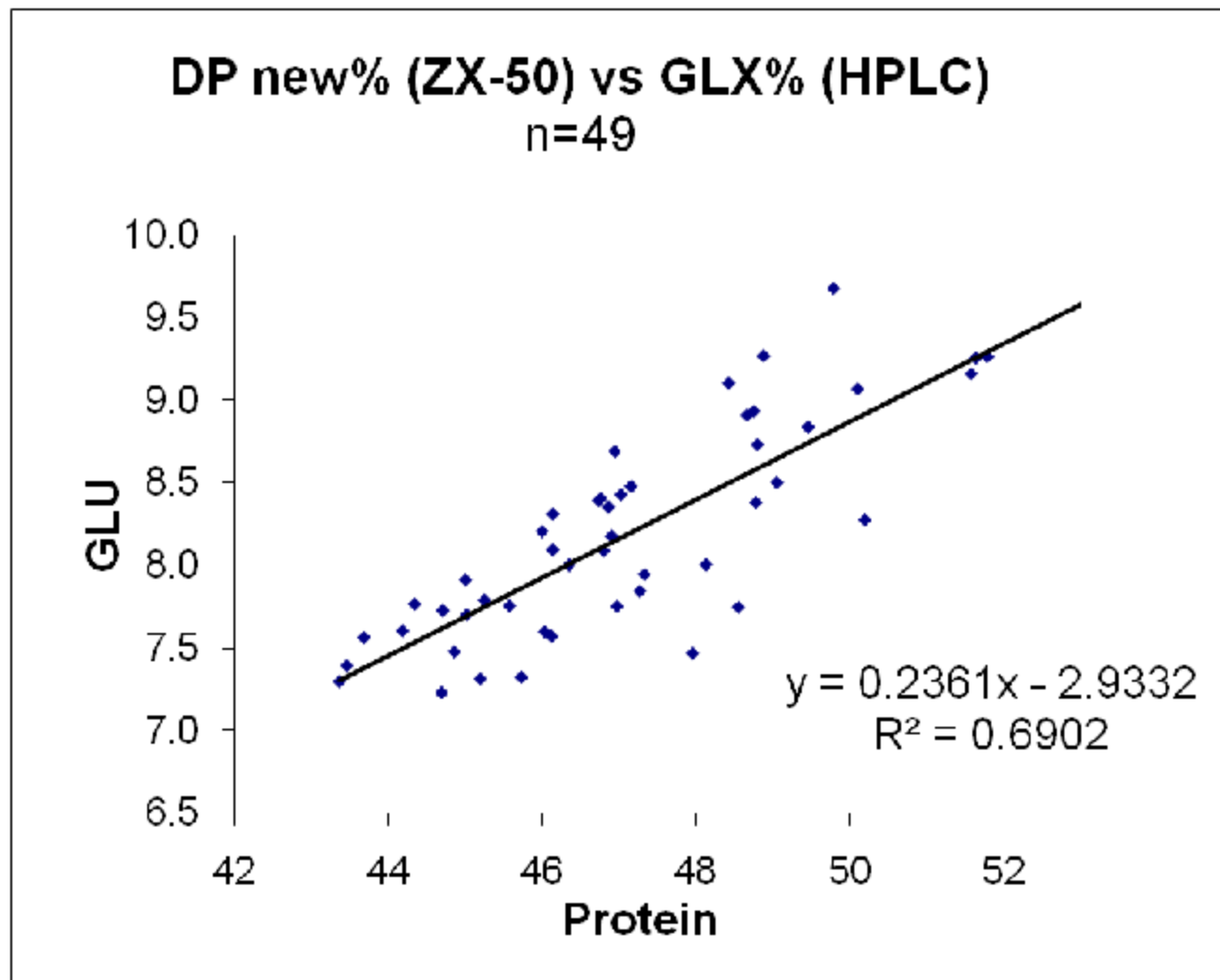


- Neutral
- Polar (uncharged)
- $\text{pK}_1 = 2.2$
- $\text{pK}_2 = 9.1$

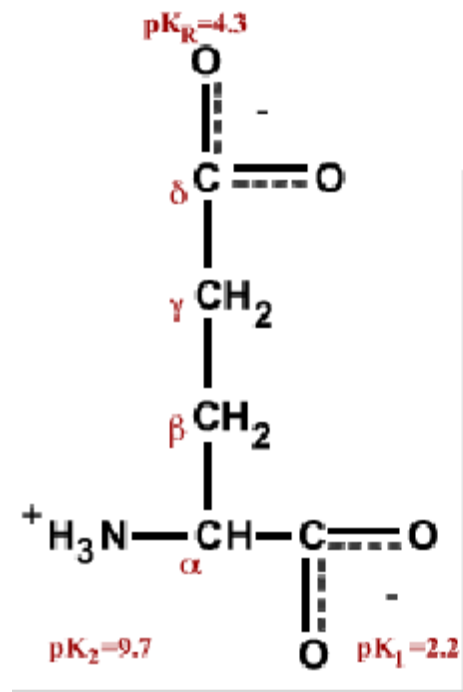
Glutamine plus Glutamic Acid, "GLX", as Total Dry Weight % vs. Dry Soybean Protein %



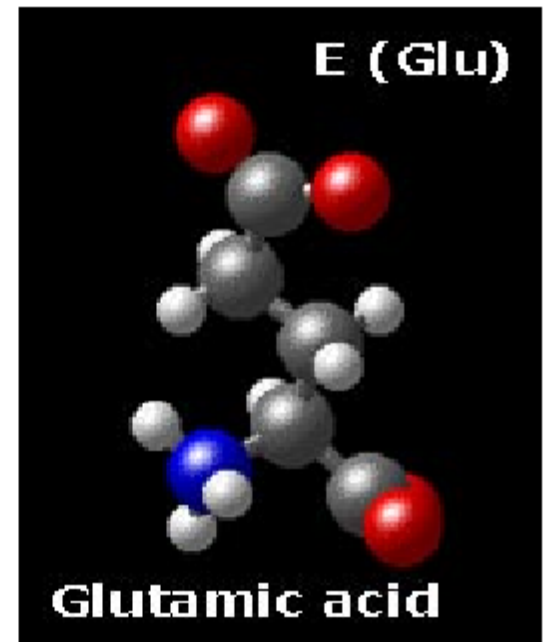
Glutamine plus Glutamic Acid, "GLX", as Total Dry Weight % vs. Dry Soybean Protein %



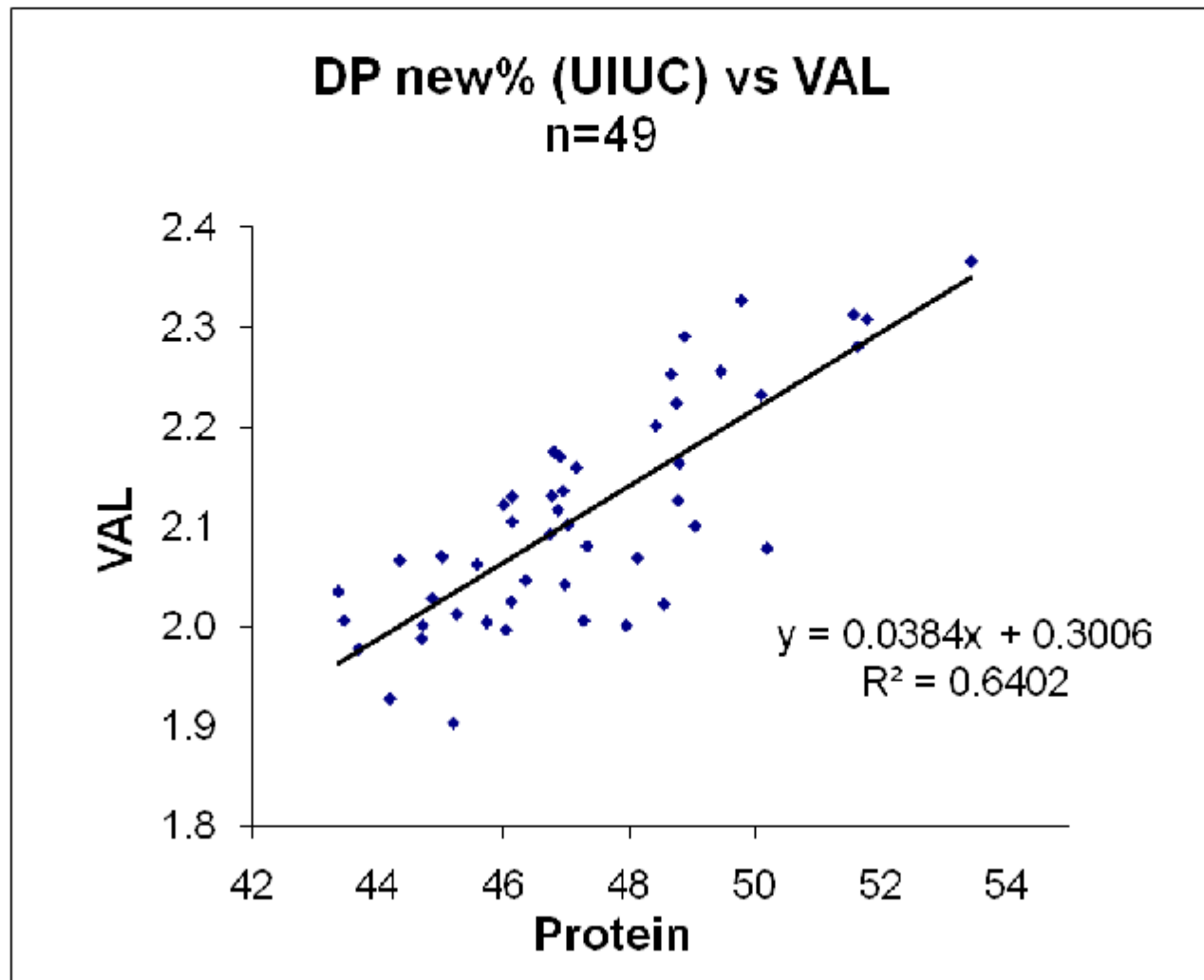
Glutamic acid



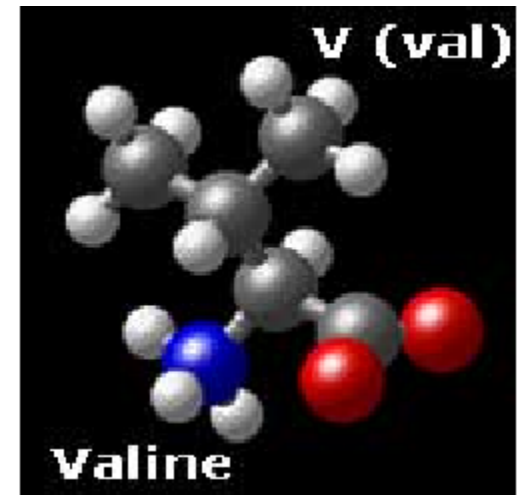
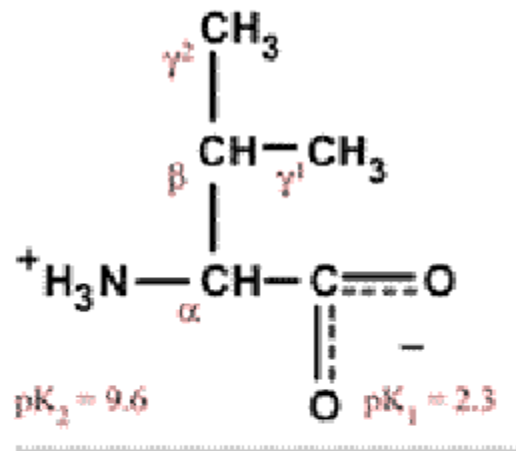
- Acidic
- Polar (charged)
- $\text{pK}_1 = 2.2$
- $\text{pK}_2 = 9.7$
- $\text{pK}_R = 4.3$



Valine

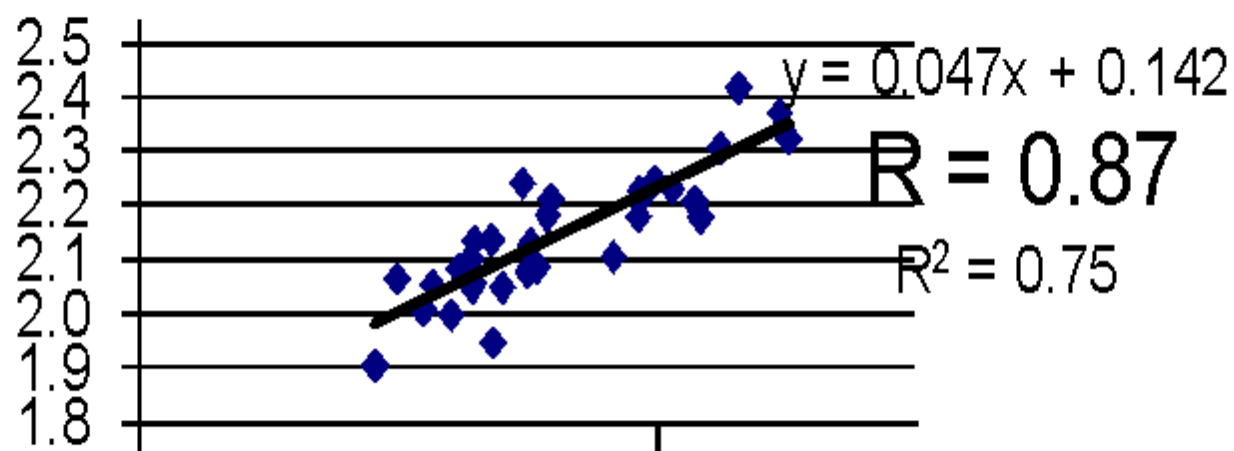


Valine



- Aliphatic
- Non-polar
- $\text{pK}_1 = 2.3$
- $\text{pK}_2 = 9.6$

PRO vs. Protein % D

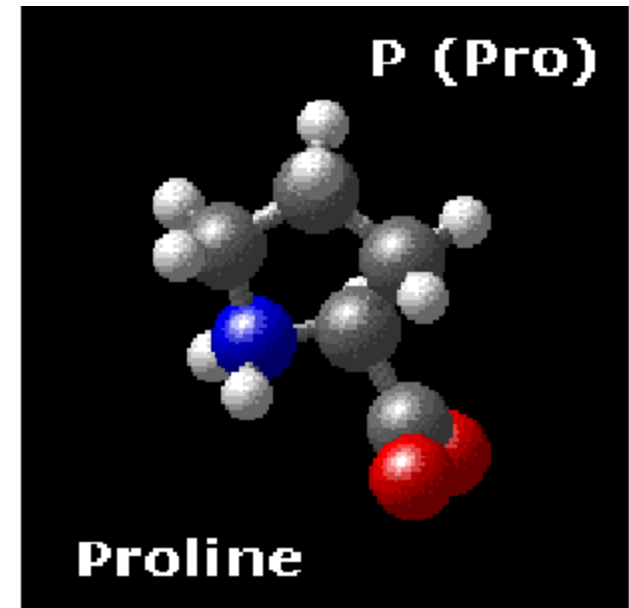
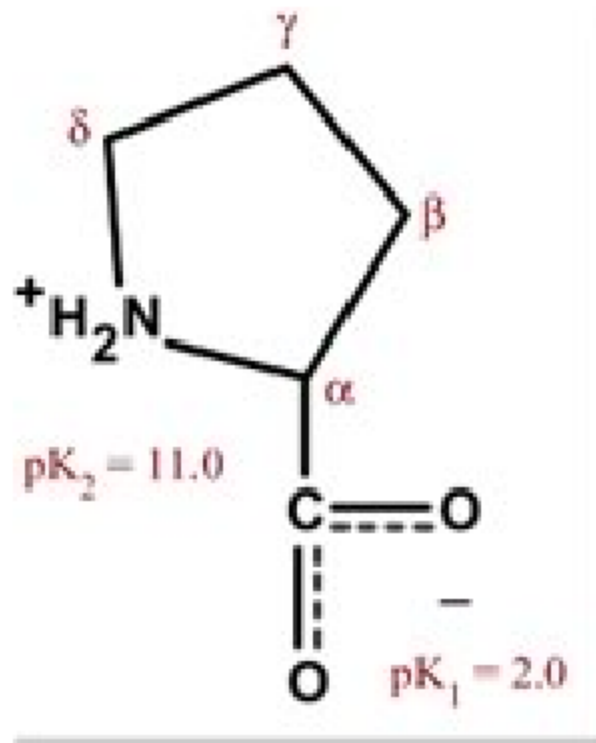


35.00

45.00

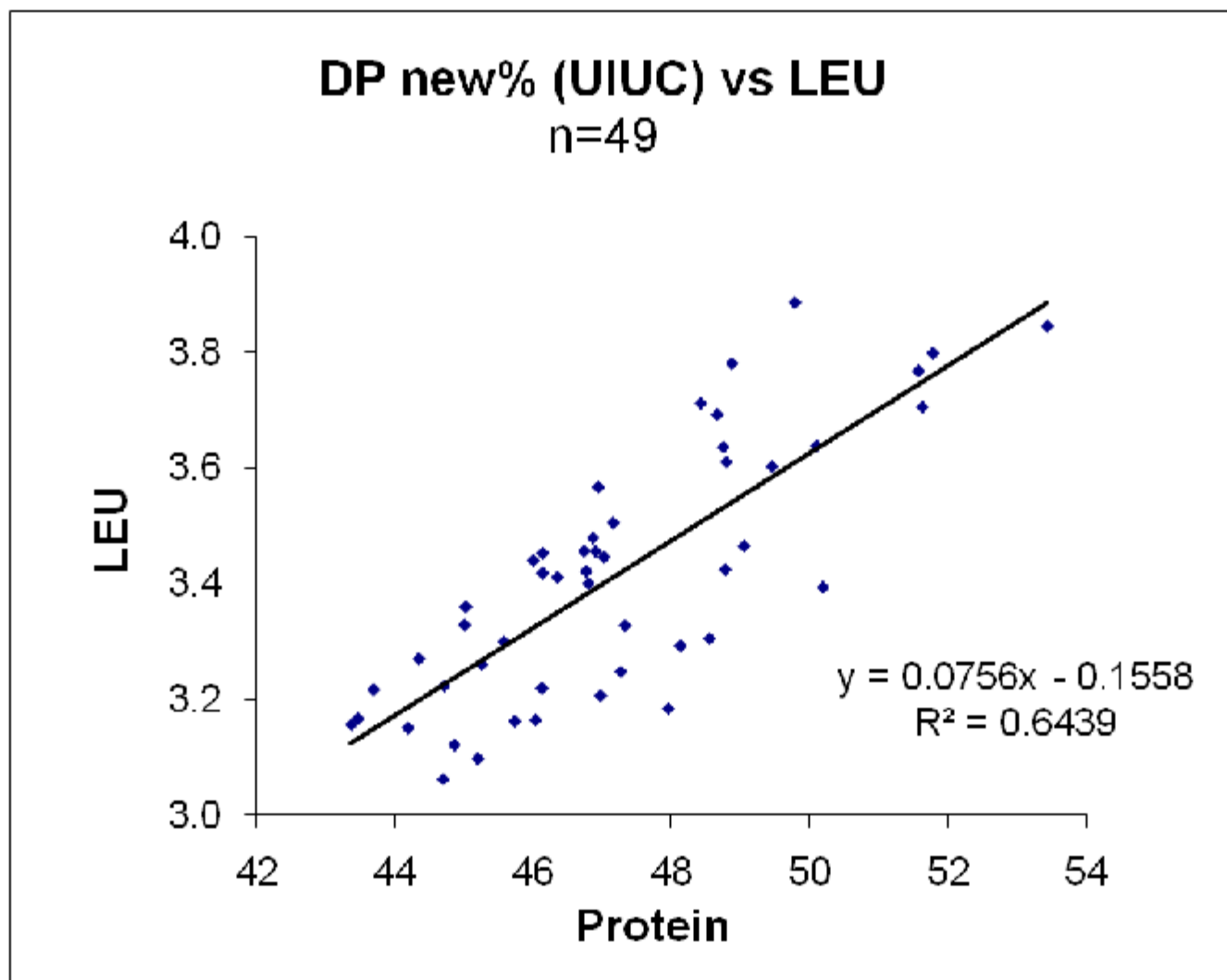
Protein by NIR

Imino Acid – Proline

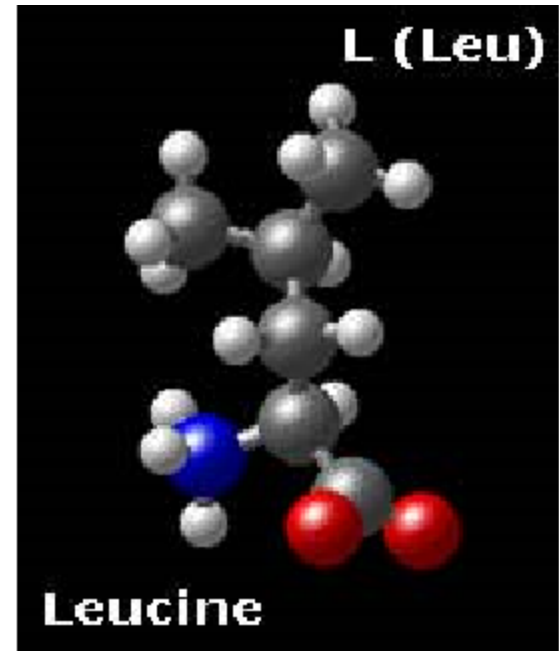
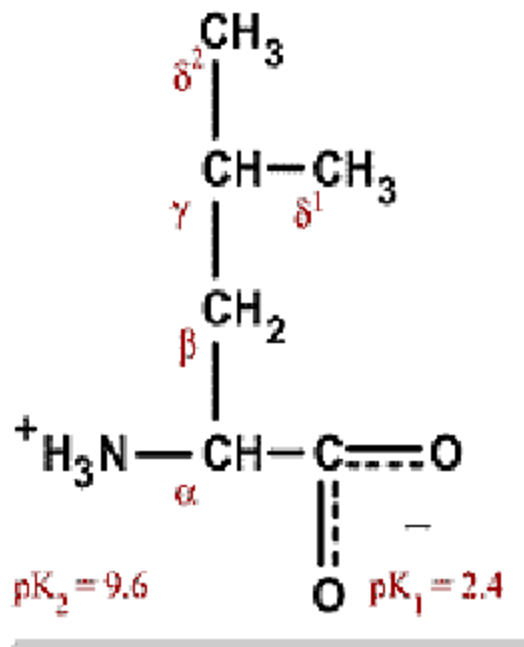


- Cyclic
- Non-polar
- Similar to aliphatic group amino acids
- $pK_1 = 2.0$
- $pK_2 = 11.0$

Leucine



Leucine



- Aliphatic
- Non-polar
- Usually buried in folded proteins
- pK₁ = 2.4
- pK₂ = 9.6

Amino Acids **Highly-Correlated** with the Dry Protein Content:

- Histidine: **R= 0.93**
- Arginine: **R= 0.90**
- Glx: **R= 0.88**
- Valine: **R= 0.87**
- Leucine: **R= 0.85**

=====

and the Imino Acid Proline: **R = 0.87**

Amino Acid Primary Data (GC-MS)

Amino Acid Primary Data:

(sample of 401 data points out of a total of 3,618)

#	ASP	THR	SER	GLU	PRO	GLY	ALA	VAL	ILE	LEU	TYR	PHI	HIS	LYS	ARG	MET	CYS	M+S
1	4.8	1.6	2.0	7.3	2.1	1.7	1.7	2.0	1.8	3.2	1.4	2.1	1.1	2.6	2.8	0.7	0.8	1.5
2	4.8	1.6	2.0	7.3	2.0	1.7	1.7	2.0	1.8	3.2	1.2	2.0	1.0	2.5	2.7	0.7	0.8	1.5
3	5.0	1.6	2.1	7.8	2.1	1.8	1.8	2.0	1.9	3.2	1.3	2.1	1.1	2.7	3.1	0.7	0.8	1.5
4	4.8	1.6	2.1	7.5	2.0	1.7	1.7	2.0	1.9	3.2	1.3	2.1	1.1	2.6	3.0	0.7	0.7	1.4
5	4.9	1.6	2.1	7.4	2.1	1.8	1.8	2.0	1.9	3.2	1.2	2.1	1.0	2.6	2.8	0.7	0.8	1.5
6	4.9	1.6	2.1	7.6	2.1	1.8	1.8	2.0	1.9	3.2	1.2	2.2	1.1	2.6	3.0	0.7	0.7	1.4
7	5.3	1.8	2.4	8.4	2.4	1.9	1.9	2.1	2.0	3.4	1.5	2.2	1.2	2.8	3.5	0.6	0.8	1.5
8	5.0	1.6	2.1	7.8	2.2	1.8	1.8	2.1	1.9	3.3	1.4	2.1	1.1	2.7	3.1	0.7	0.8	1.5
9	5.1	1.7	2.3	7.9	2.2	1.9	1.9	2.1	2.0	3.3	1.3	2.2	1.1	2.7	3.1	0.7	0.9	1.7
10	5.7	1.4	2.7	9.1	2.3	2.1	2.0	2.2	2.2	3.6	1.6	2.4	1.2	3.0	3.7	0.8	1.0	1.9
11	6.2	1.6	2.9	9.7	2.5	2.2	2.6	2.3	2.2	3.9	1.6	1.8	1.3	3.1	4.1	0.9	1.0	1.9
12	6.1	1.7	2.8	9.5	2.5	2.2	2.1	2.4	2.3	3.8	1.7	2.5	1.4	3.2	4.2	0.9	1.1	1.9
13	5.9	1.5	2.8	9.3	2.4	2.1	2.0	2.3	2.2	3.8	1.6	2.5	1.2	3.1	3.8	0.9	1.1	1.9

15	6.0	1.5	2.8	9.2	2.3	2.1	2.1	2.3	2.2	3.8	1.5	2.4	1.3	3.0	4.1
16	5.7	1.5	2.7	8.8	2.4	2.0	2.0	2.3	2.0	3.6	1.6	2.3	1.2	2.9	4.0
17	6.0	1.5	2.8	8.7	2.3	2.0	2.0	2.2	2.1	3.6	1.6	2.4	1.2	2.9	3.9
18	5.9	1.5	2.7	9.3	2.4	2.1	2.1	2.3	2.2	3.7	1.6	2.4	1.3	3.1	3.9
19	5.7	1.8	2.5	8.9	2.4	2.0	2.0	2.2	2.1	3.6	1.6	2.4	1.2	3.0	3.8
20	5.8	1.4	2.8	9.1	2.6	2.0	2.0	2.2	2.2	3.7	1.6	2.4	1.4	3.0	3.8
21	5.9	1.8	2.6	9.3	2.5	2.1	2.0	2.3	2.2	3.8	1.6	2.2	1.2	3.0	3.8
22	5.7	1.8	2.5	8.9	2.4	2.1	2.0	2.3	2.1	3.7	1.5	2.4	1.2	3.0	3.8
23	5.6	1.7	2.3	8.5	2.2	1.9	1.9	2.1	2.0	3.5	1.5	2.3	1.2	2.8	3.7
24	5.3	1.4	2.6	8.2	2.2	1.9	1.9	2.1	2.0	3.4	1.5	2.2	1.2	2.8	3.5
25	5.4	1.6	2.4	8.4	2.3	1.9	1.9	2.1	2.0	3.4	1.4	2.2	1.1	2.7	3.5

Amino Acid ROVs

AA	ASX	THR	SER	GLX	PRO	GLY	ALA	VAL	ILE
Max	6.20	1.82	2.90	9.67	2.60	2.18	2.57	2.37	2.27
Min	4.73	1.35	2.04	7.23	1.95	1.72	1.72	1.90	1.78
Ratio	0.24	0.26	0.30	0.25	0.25	0.21	0.33	0.20	0.22

AA	LEU	TYR	PHE	HIS	LYS	ARG	MET	CYS	met+cys
Max	3.89	1.66	2.53	1.36	3.20	4.67	0.88	1.08	1.95
Min	3.06	1.11	1.54	1.00	2.52	2.73	0.65	0.72	1.38
Ratio	0.21	0.33	0.39	0.26	0.21	0.41	0.27	0.34	0.29
Legend:		Red (poor; low)		Green (acceptable)		Blue (good; high)			

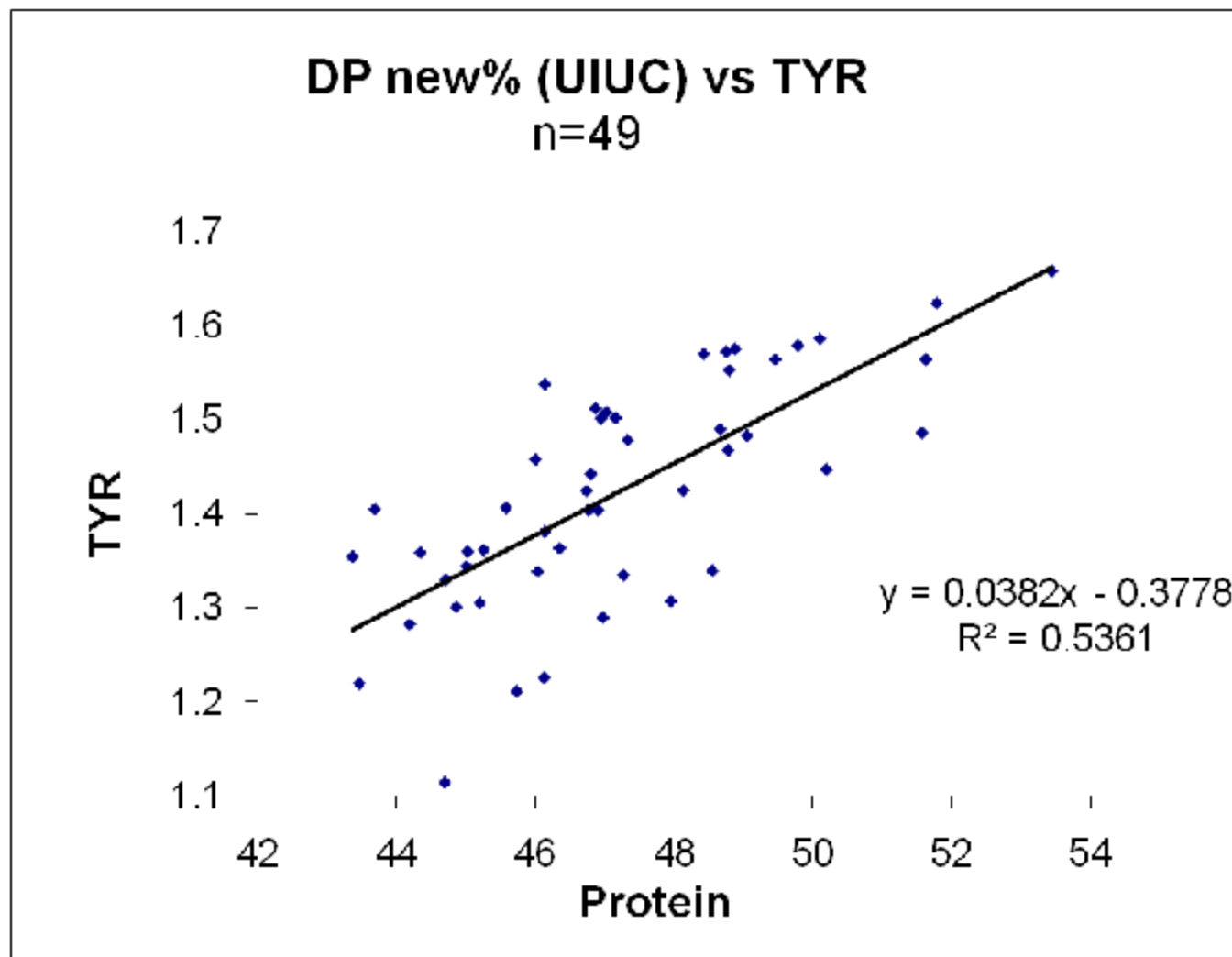
SOY PROTEIN and MOISTURE DATA , with Soybean Accession Identifiers

ID v = very, lt = light, grn = green,
brn = brown, bl = black

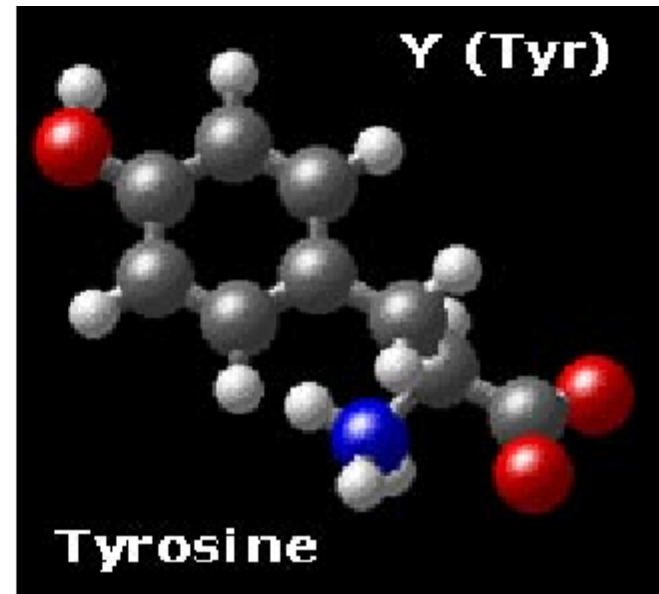
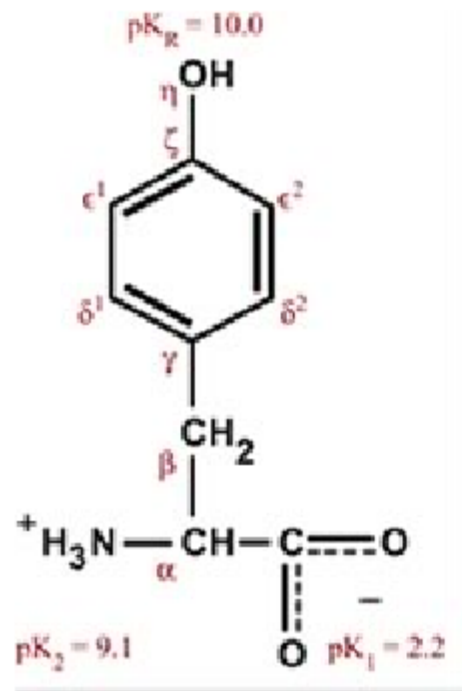
acid	coat color	cultivar	seed
source			
PI536636 Y	Ripley	93U-5051	
PI548518 Y	Cutler 71	92U-901	
PI548477 Lgn	Ogden	99S-4040	
PI548379 Y	Mandarin	00U-109	
PI548603 Y	Perry	98U-1459	
PI533655 Y	Burlison	95U-2238	
PI548311 Y	Capitol	97U-2231	
PI548659 Y	Braxton	94S-6	
PI567551 ltGn,1/10 blk	Huang li	94U-785	
PI458057 Dk Gn		96U-2173	
PI567704 Y, 1/2 brn	Fu yang (23)	93U-2482	

VIP-Data			Example:	
10DataPoints	TOTAL: 3,816data-points			
Prot	Moist	Oil	DP	DP calc
	DO calc			
35.57	11.10	20.50	40.01	40.01
	23.06			
36.91	10.00	20.76	41.00	41.01
	23.07			
41.75	10.41	17.06	46.60	46.60
	19.04			
41.44	10.30	10.02	46.20	46.20
	11.17			
41.10	10.16	18.60	45.75	45.75
	20.70			
36.71	9.12	20.60	40.40	40.39
	22.67			
38.03	10.84	20.24	42.65	42.65
	22.70			
39.52	10.44	19.34	44.13	44.13
	21.59			
40.17	9.04	18.80		44.16
	20.67			
40.67	10.25	18.30	45.31	45.31
	20.39			

Tyrosine vs. %DP

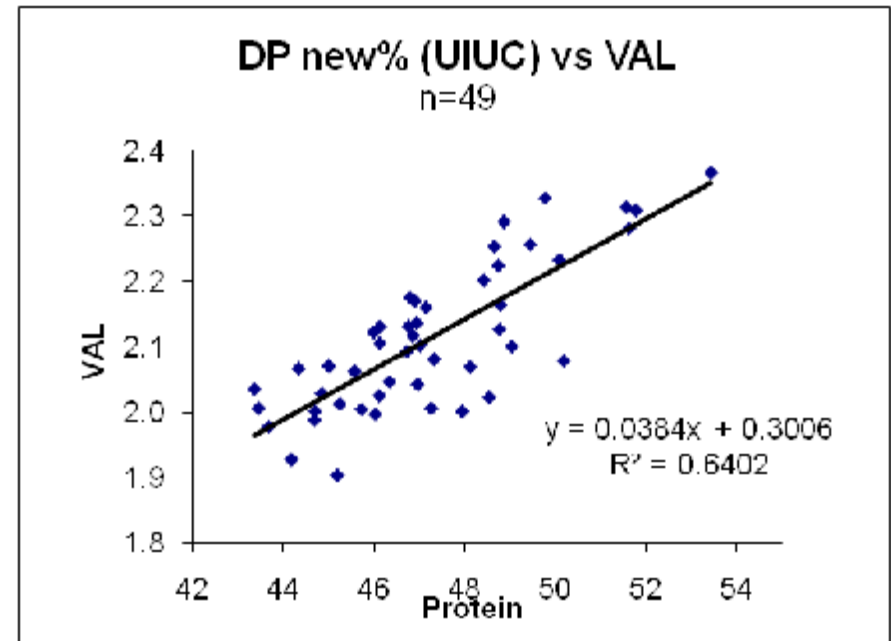
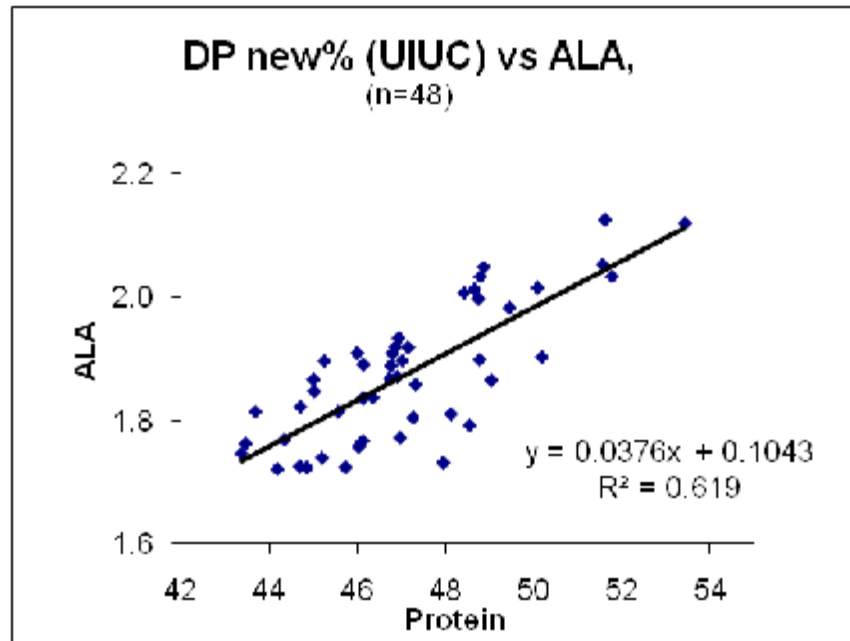


Tyrosine

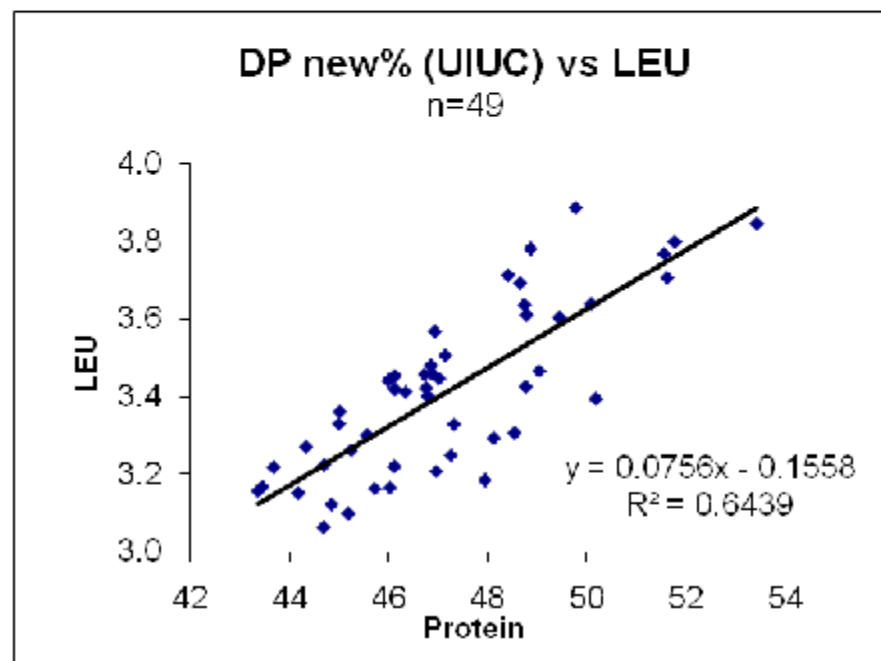
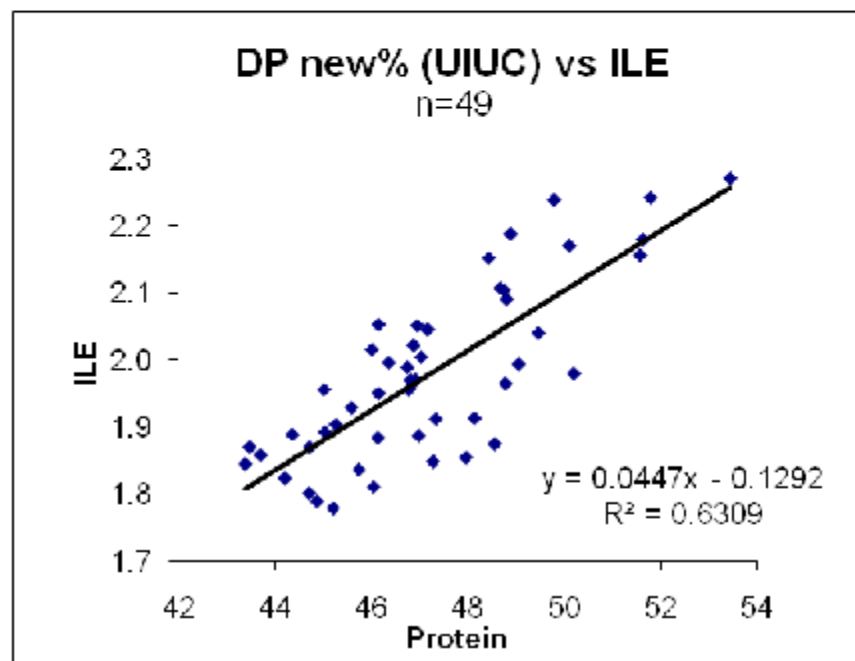


- Aromatic
- Non-polar
- Not as hydrophobic as Phe
- $\text{pK}_1 = 2.2$
- $\text{pK}_2 = 9.1$

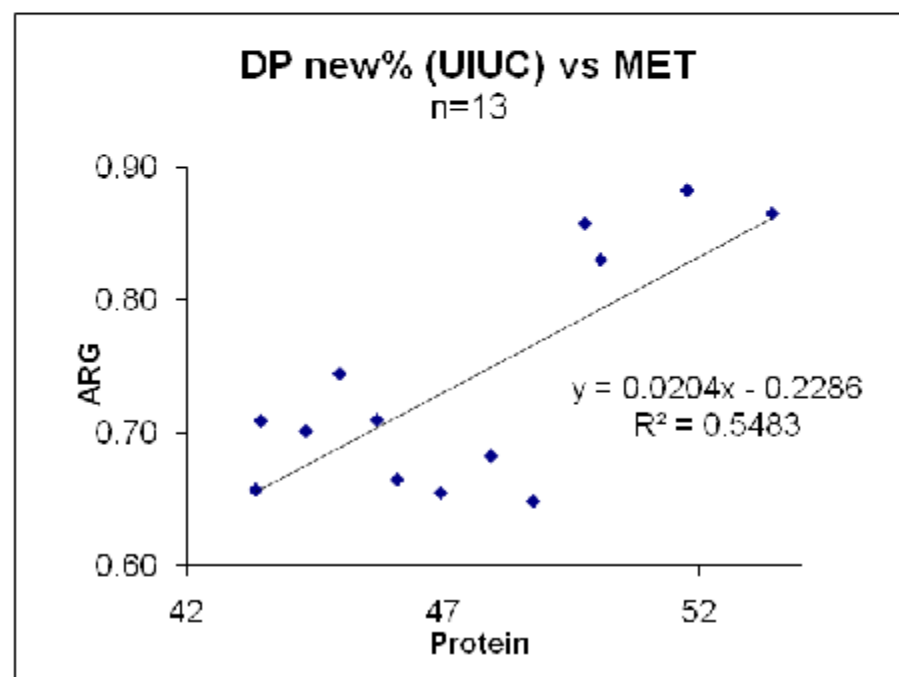
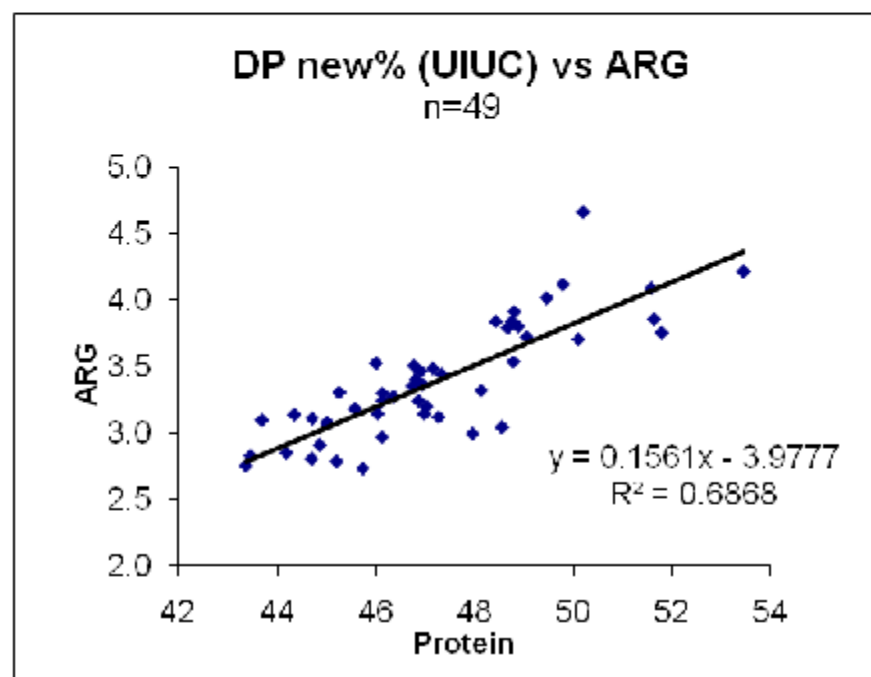
Amino Acid Primary Data (GC-MS), cont'd



Amino Acid Primary Data (GC-MS), cont'd



Amino Acid Primary Data (GC-MS), cont'd



Amino Acid Summary

- Total protein measurement is by wet chemistry analysis, for which a ~96% correlation with our lab's NIR analysis is **confirmed**.
- Some of the amino acid correlations depend on the type of amino acid residue.
- There is a potential for selecting the composition of amino acids seen in soybeans.

Amino Acids,

p.2

- A few amino acid residues vary much more across some soybean varieties than others
- The combined NIR, NMR and GC-MS data for amino acids in soybean seeds shows the possibility of generating reliable calibrations for selected triplet groups of amino acids using FT-NIR Spectroscopy.
- NIRS data for AA can be validated and may thus become a very useful tool for cross-breeding and genetic selection purposes.

3. Proposed Research Methods

I. Techniques, Data analysis and Expected Results

- Primary and secondary techniques ; Chemical and Hyperspectral NIR Imaging ; Fluorescence Correlation Spectroscopy and Microspectroscopy)
- Data Analysis: Principles of NIRS, Data Corrections, Regression Algorithms and Multivariate analysis.

I. Limitations and Advantages of proposed methods

II. Tentative schedule to conduct major steps

I. Techniques, Data analysis and Expected Results

A. TECHNIQUES:

- NIR Spectroscopy,
- GC-MS
- HPLC and IEC
- Chemical Imaging,
- Fluorescence Correlation Spectroscopy

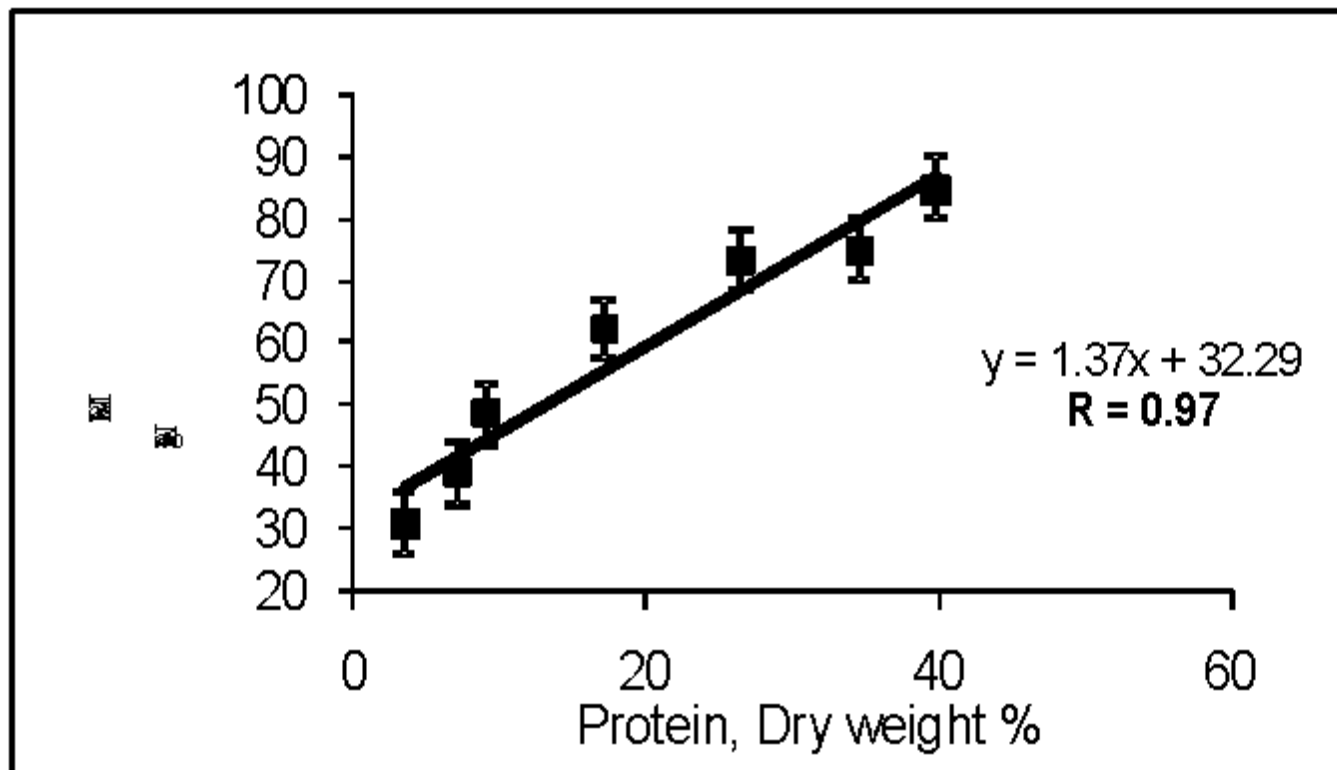
B. Data Analysis

C. Expected Results

NIR Analysis of Amino Acids & Proline in Soybeans

- Wet chemistry analysis :
 - Ion Exchange Chromatography (IEC) / UV / vis Abs. / Fluorescence
 - Derivatization HPLC
 - GC-MS (USDA — Peoria)
- NMR as Primary Method

Soybean Proteins Content by C-13 NMR and Sigma Methods



Source: Baianu, You, Costescu, Prisecaru & Nelson. AOCS Proc., (2005)

Principles of NIR Spectroscopy

- IR absorption spectra occur because the atom-to-atom bonds within molecules can vibrate and rotate thus generating series of different energy levels among which rapid transitions can occur.
- According to Quantum Mechanics, the vibro-rotational energy levels of a molecule can be approximated by the following equations:

$$E_{NIR} = E_{rot} + E_{vib} + E_{anh} = j(j+1)Bhc + [1 - x(n+1/2)]h\nu$$

- where: **j** : rotation quantum number = 0,1,2, ...
 - **n**: vibration number = 0,1,2,...;
 - **E**: Energy eigenvalues , and
 - **x**: anharmonicity constant (~0.01).

Current Near-Infrared Instruments: Techniques

Current NIR instruments utilize EM radiation with wavelengths from ~ 750 nm to 2500 nm. Their operation is based on the fact that molecular bonds stretch and/or bend, thus causing absorption bands at certain characteristic IR and NIR wavelengths that are proportional to the amount of the absorbing components present in the sample, e.g., amide 1 and 2 bands.

Apparent Absorbance



- The calculated absorbance is usually referred to as the “**apparent absorbance**,” and it can be significantly affected by **Specular Reflection and Light Scattering**, even for thin samples.
- Therefore, in order to obtain reliable NIR quantitation, **Spectral Pre-Processing and Corrections** are always required.

Data Correction Problems

- We found that the NIR methods currently employed in industry for:
 - spectral pre-processing
 - correction of light scattering
 - specular reflection effects

are in need of substantial improvements in order to produce high accuracy, robust and stable calibrations for rapid composition analyses of seeds.

Light Scattering Corrections for Soybean NIR Spectra

Spectral variations between soybean samples can be caused by:

- chemical composition differences
 - (i.e., what you want to measure)
- Spurious effects*
 - specular reflection
 - scattering effects
 - internal reflection

*These do not monitor chemical composition -- and are therefore measurement artifacts that are undesirable and distort the data.

Fourier Transform NIR

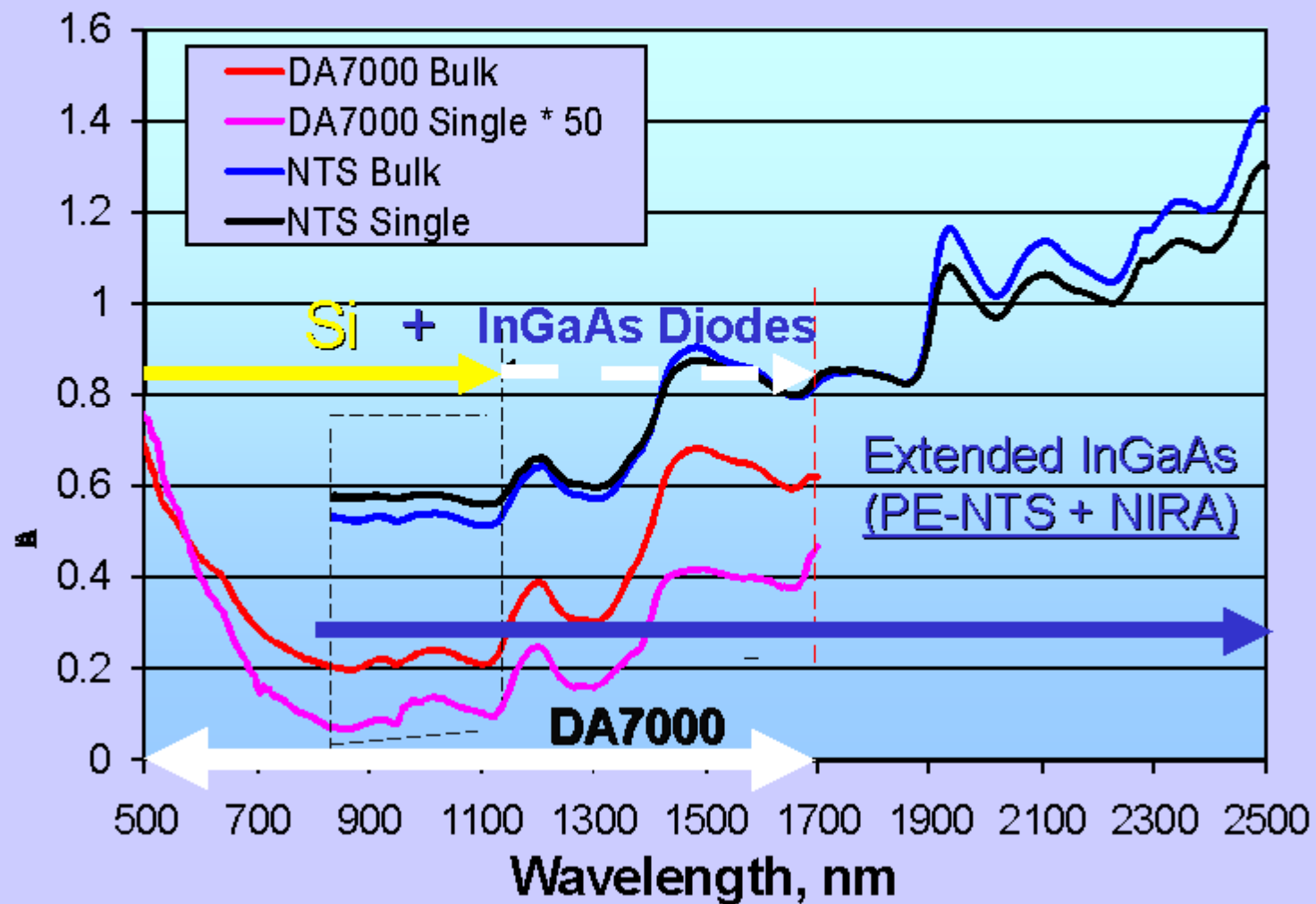
- Recent NIR Spectroscopy techniques utilizing Fourier Transform (FT) fulfill all these conditions, but require pre-calibration by AOCS-approved wet chemistry techniques, using well-defined and stable sample standard sets of 50 to 100 different samples.

FT-IR Spectrometer *Spectrum One* and FT-NIR Spectrometer *SpectrumOne-NTS*

- Introduced in 2001 by Perkin Elmer Co. (Shelton, CT, USA) for High Sensitivity, high-resolution and long-term stability
- *SpectrumOne* and *SpectrumOne NTS* have a similar look but are configured for different spectral ranges (e.g., IR and NIR, respectively).



Comparison of Soybean Spectra Collected with either Perten DA7000 or the PE Spectrum One NTS (with Extd InGaAs/NIRA) NIR Instruments



Lambert-Beer's Law

- Absorption is a universal spectroscopic phenomenon that has immediate chemometric applications, because it is directly related to the constituent concentration as described by:

Lambert-Beer's Law, which states that ...

$$A = \epsilon * C * L$$

where:

A = True Absorbance

= Extinction coefficient of analyte

= **Concentration**

= Pathlength of light



Lambert-Beer's Law, Con't

- The absorbance of a sample is difficult to measure directly
- In practice, the absorption is often calculated indirectly from the measurement of the reflectance ($A = \text{Log } 1/R$), or transmittance ($A = \text{Log } 1/T$), that can be readily measured even for thick samples, provided these do not possess a composite structure, such as thick, multiple layers with different compositions
- The calculated absorbance is usually referred to as the 'apparent absorbance,' and it can be significantly affected by Specular reflection and light scattering even for thin (e.g., 5 mm) samples.
- Therefore, in order to obtain reliable NIR quantitation, spectral pre-processing and corrections are always required

Light Scattering Corrections for Soybean NIR Spectra

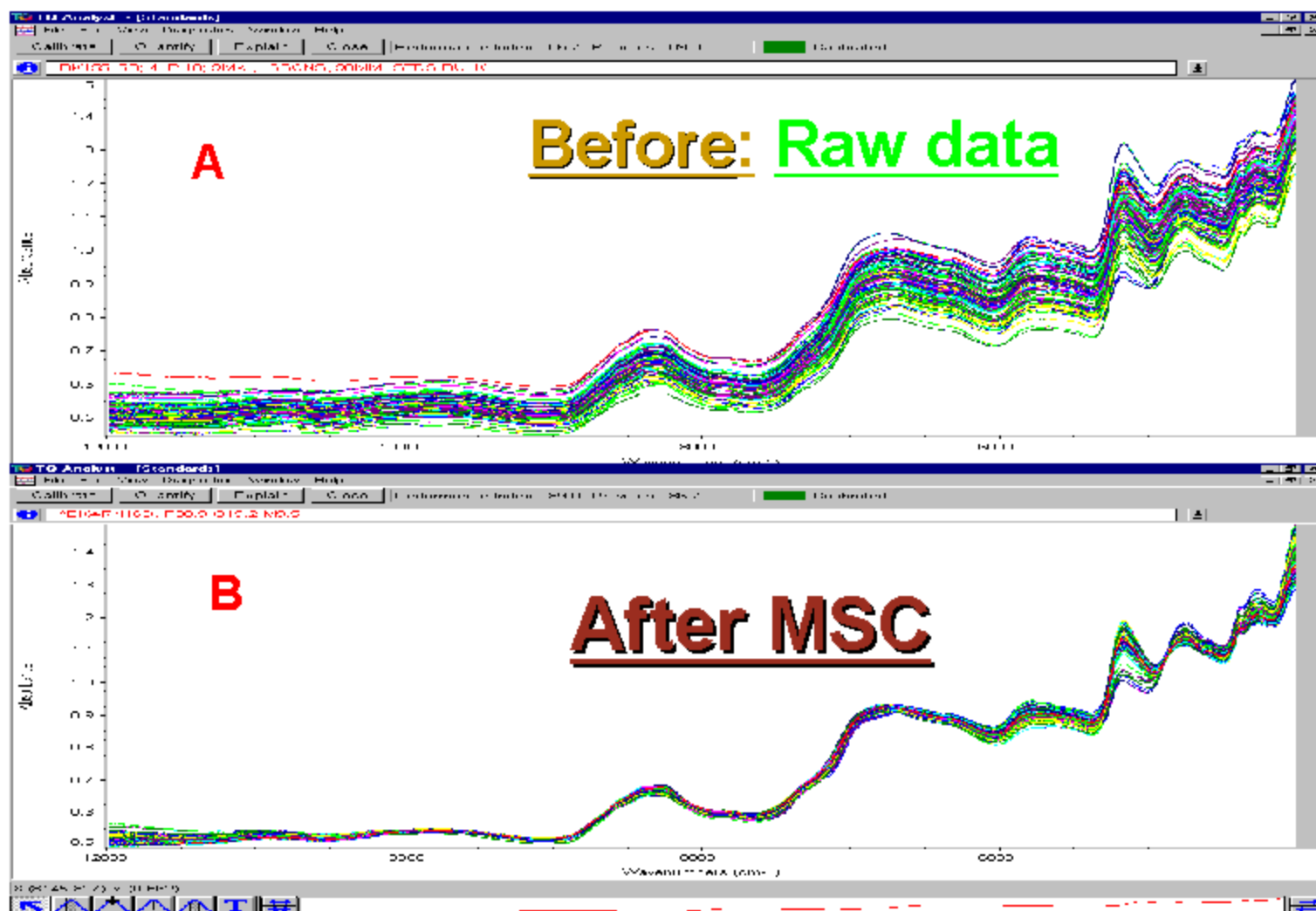
- Spectral variations between soybean samples can be caused by chemical composition differences, specular reflection, as well as light scattering effects (that do not monitor chemical composition —and are therefore a measurement artifact)
- The effects of **light scattering** and/or **specular reflection** on the NIR spectra of soybean need be investigated, and eliminated if at all possible

NIR Light Scattering Corrections, p.2

One finds that the methods that are currently employed by the NIR industry for spectra pre-processing and corrections of light scattering and/or specular reflection effects are in need of substantial improvements in order to produce calibrations that are:

- Highly accurate
- Robust
- Lead to stable calibrations for rapid composition analyses of seeds

SpectrumOne NTS Spectra of Bulk Soybean Samples, before (A) and after (B) Multiple Scattering Correction (MSC)



Detrimental Effects of Light Scattering on the Accuracy of NIR Analysis for Whole Soybeans

(measured with the FT-NIR, SpectrumOne NTS Spectrometer)

Component	R		SECV	
	No MSC	MSC	No MSC	MSC
Protein	99.5	99.9	0.63	0.26
Oil	99.3	99.9	0.29	0.13
Moisture	99.8	99.9	0.26	0.17

R: Correlation coefficient

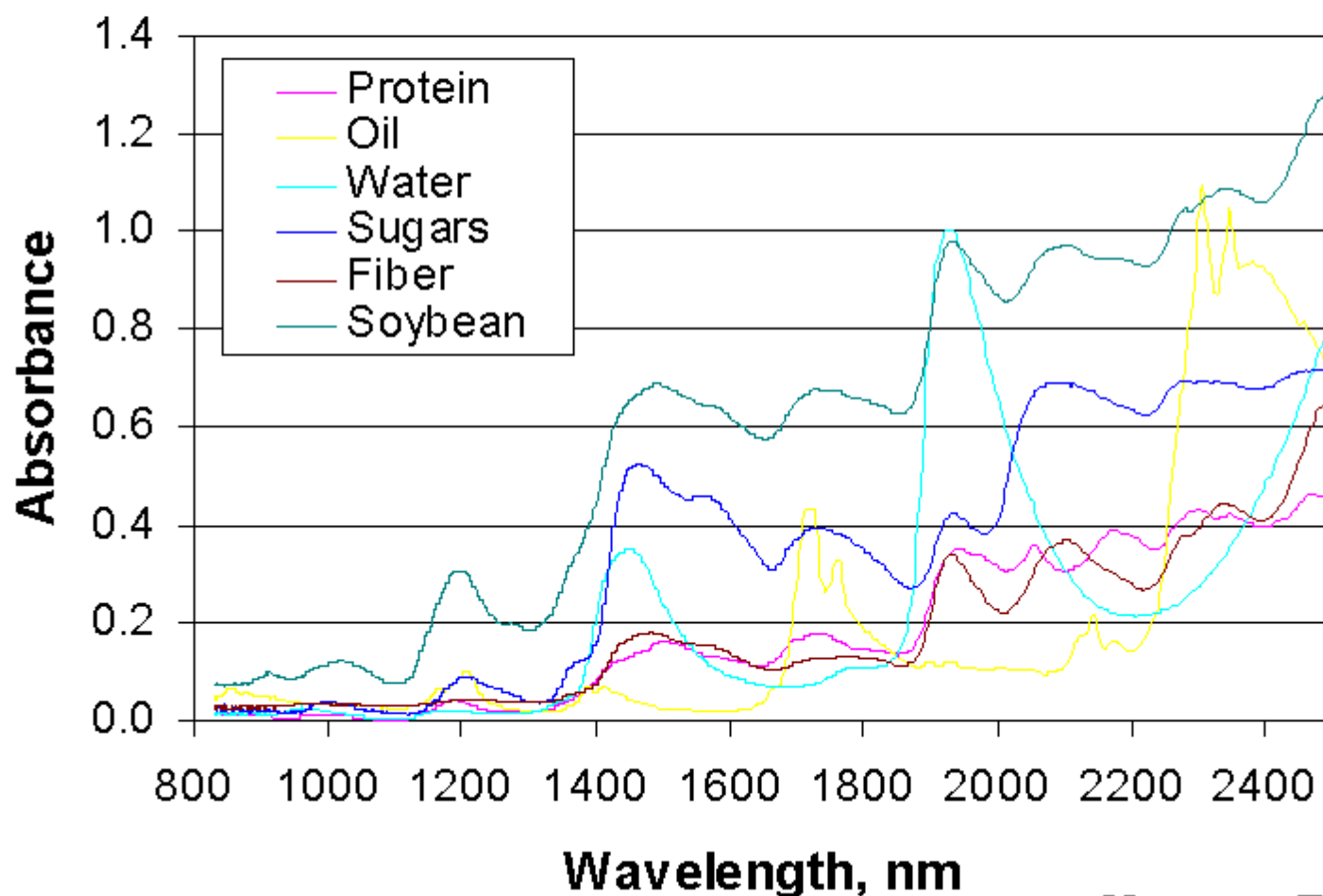
SECV: Standard Error of Cross Validation

(Tested with 65 bulk whole soybean standards)

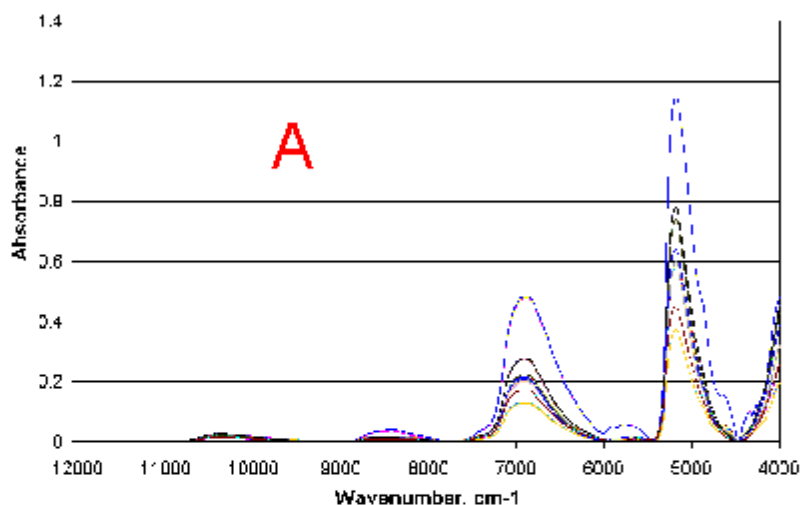
Source: T. You, 2006

FT-NIR Spectra of Five Major Soybean Components

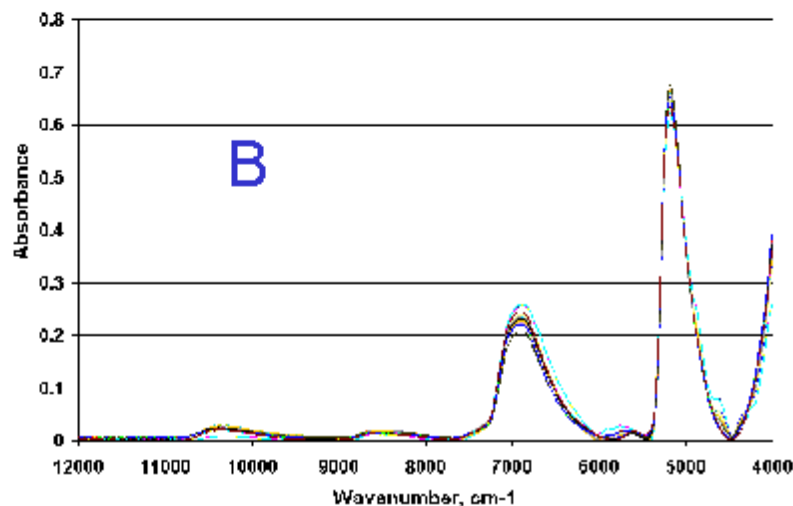
Collected on the Perkin-Elmer SpectrumOne NTS FT-NIR Spectrometer



SpectrumOne NTS FT-NIR Spectra of Soy Protein Isolates (SPI) in H₂O, before (A) and after (B) Multiple Scattering Correction (MSC) (Source: I.C. Baianu et al., 2009)



Before MSC



After MSC

Component	Number of Factors		R		SECV	
	NoMSC	MSC	NoMSC	MSC	NoMSC	MSC
SPI	6	6	0.998	0.999	0.87	0.54
H2O	6	6	0.998	0.999	0.87	0.54

R: Correlation coefficient

SECV: Standard Error of Cross Validation

Source: T. You, 2006

SECV

The Standard Error of Cross Validation (SECV) is defined as:

$$SECV = \sqrt{1/n \times \sum_{i=1}^n (y_i - \hat{y}_i)^2}$$

where n is the total number of samples, y_i denotes the standard value of the component concentration, \hat{y}_i and denotes the predicted component concentration

SEP

The **standard error of prediction** is the standard deviation of the sample mean estimate of a population mean.

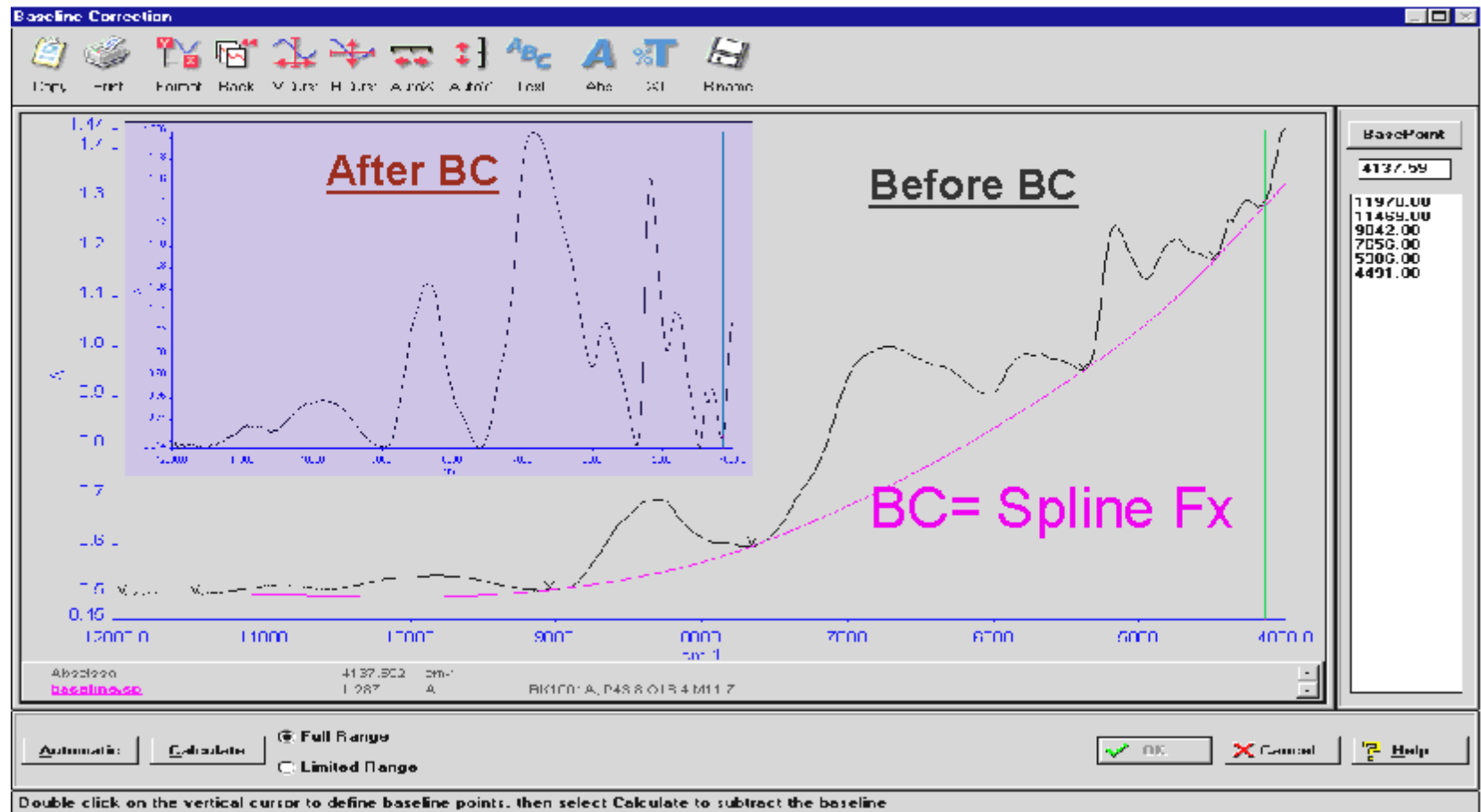
$$SE_{\bar{x}} = \frac{s}{\sqrt{n}}$$

Usually estimated by the sample estimate of the population standard deviation (sample standard deviation) divided by the square root of the sample size:

where

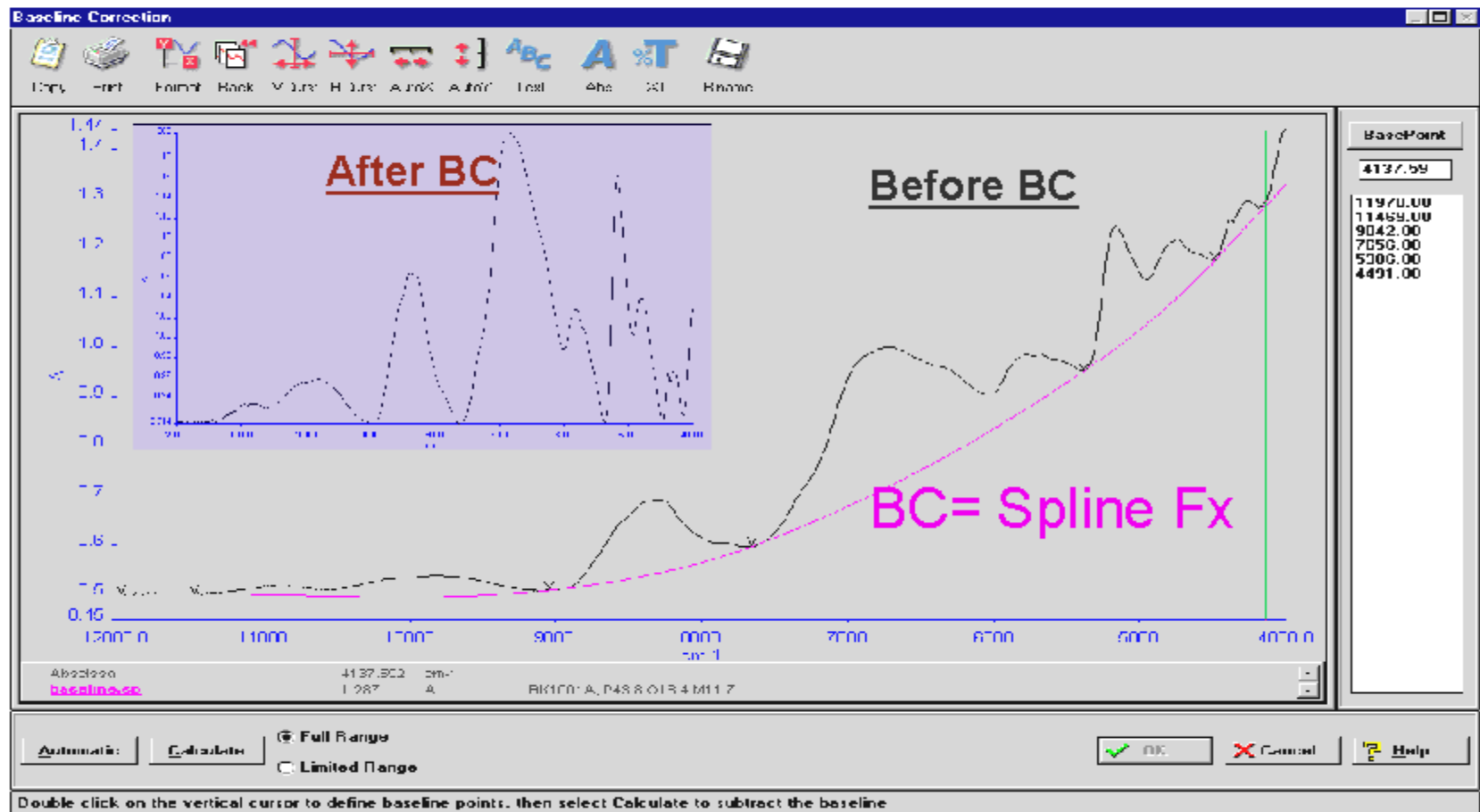
s is the sample standard deviation (i.e. the sample based estimate of the standard deviation of the population), and
 n is the size (number of observations) of the sample.

Illustration of the Interactive, Spline Baseline Correction (BC)* of the FT-NIR Spectrum of a Whole Soybean Seed, with Coat



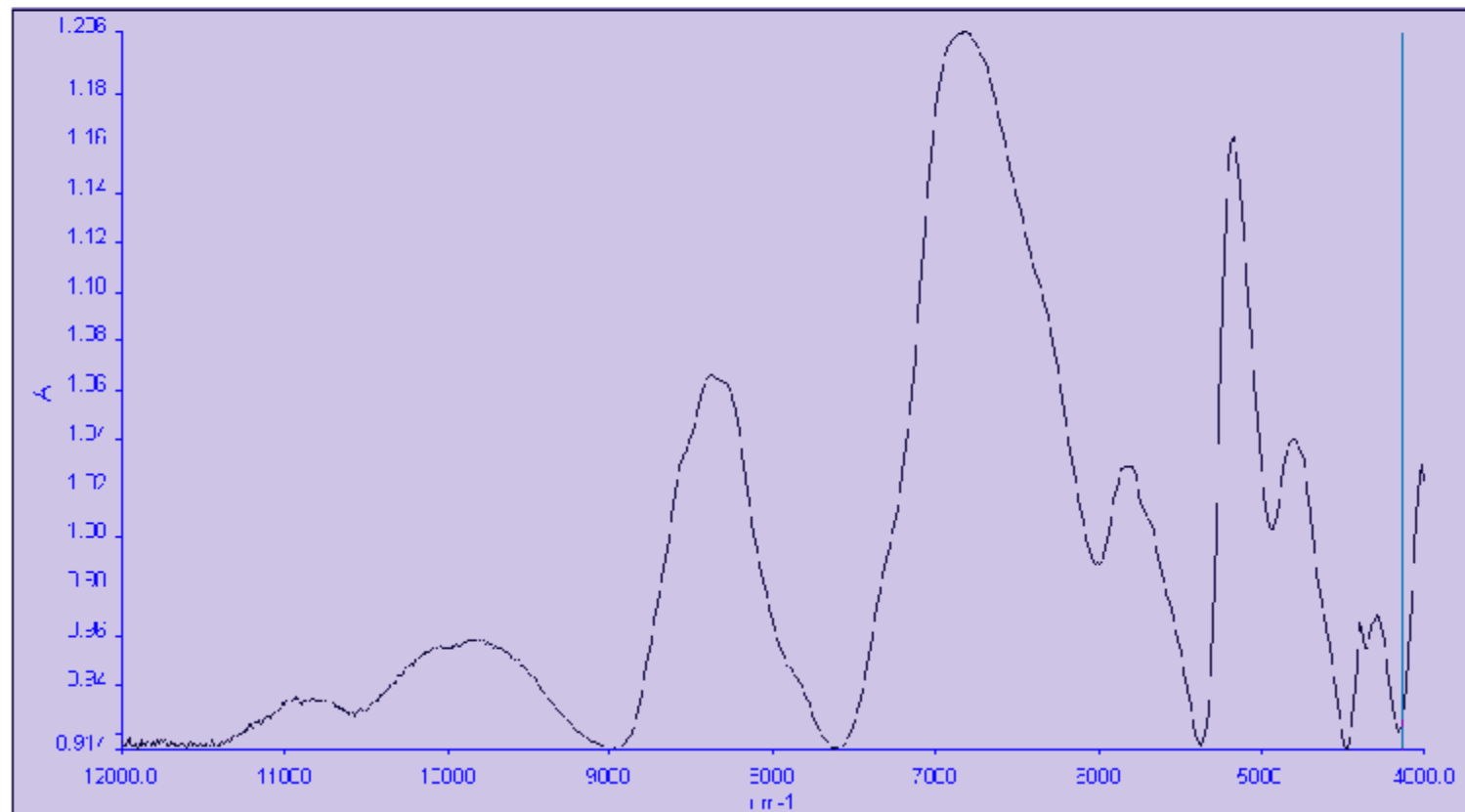
•Note: Only the PE Spectrum program supports this interactive, spline-function, Fx, baseline correction (BC), shown above in purple color.

Illustration of the Interactive, Spline Baseline Correction (BC) * of the FT-NIR Spectrum of A Whole Soybean Seed, with Coat



•**Note:** Only the PE Spectrum program supports this interactive, spline-function, Fx, baseline correction (BC), shown above in purple color.

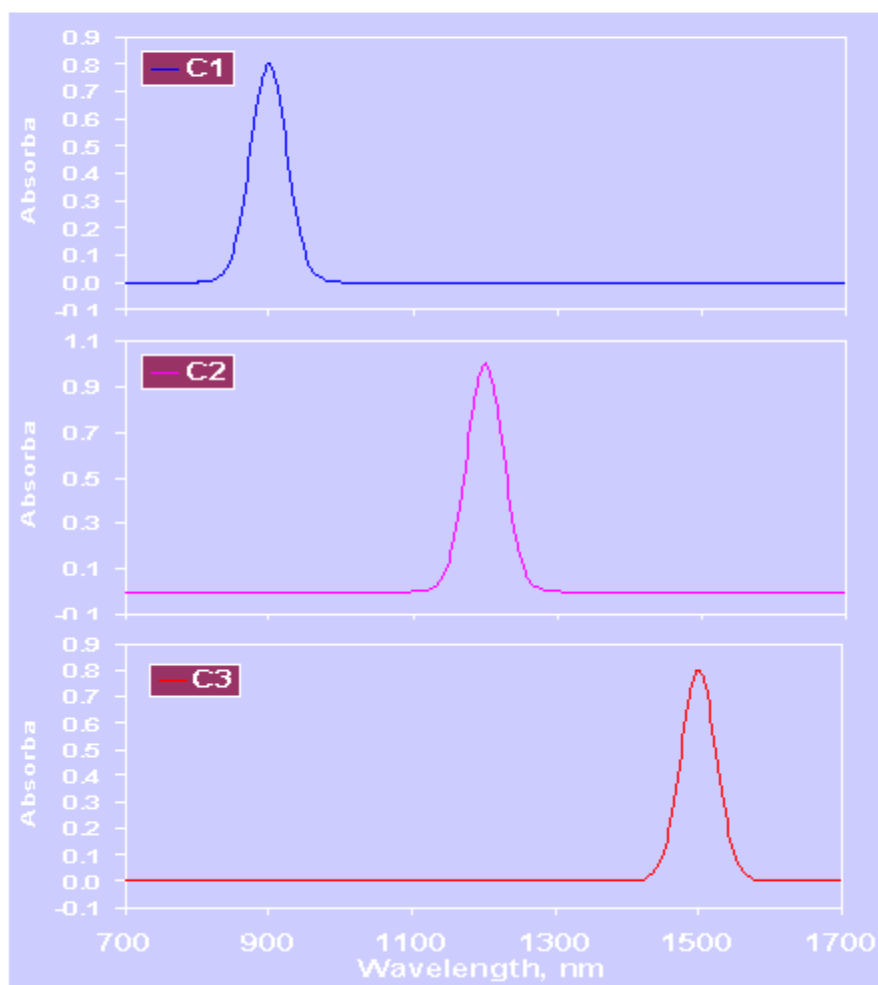
True FT-NIR Absorption Spectrum of Soybeans with Coat



Calibration

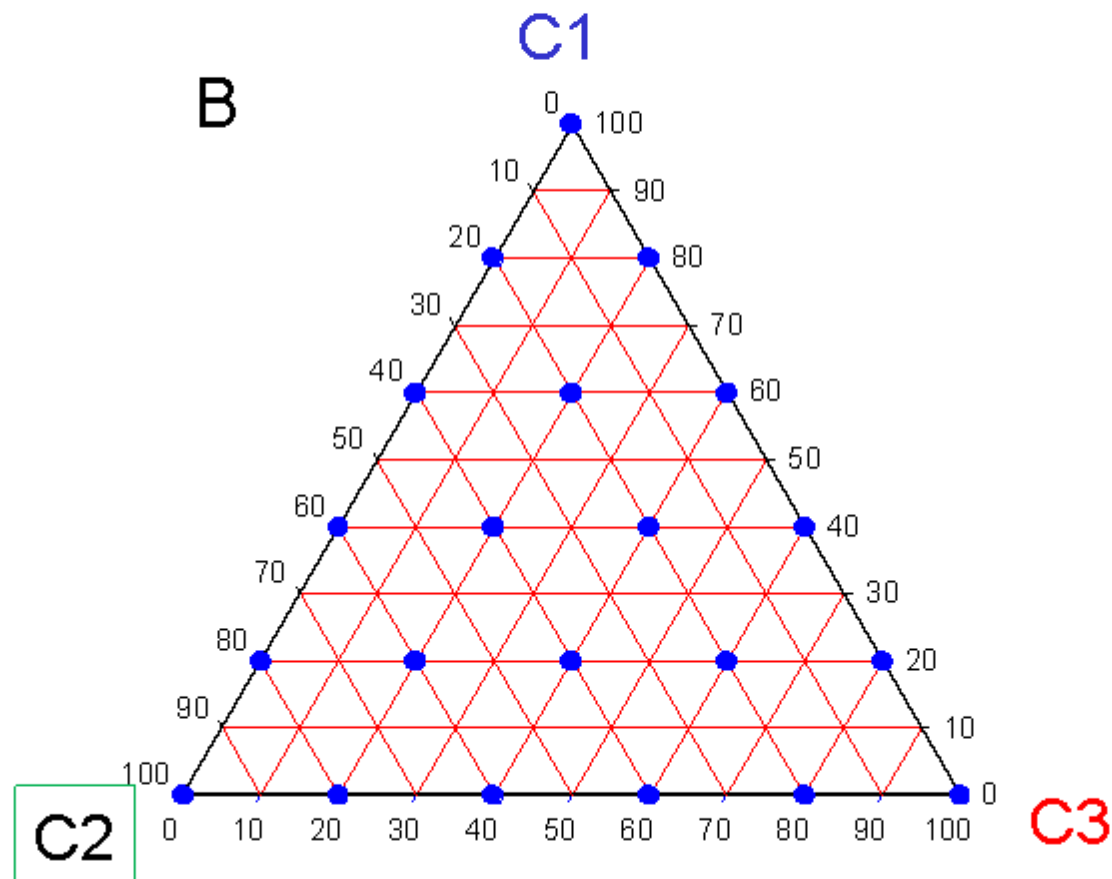
- Generate or Select a suitable set of Standard Samples of Known composition
- Obtain Raw FT-NIR data
- Correct Data for Multiple Scattering
- Use Lambert-Beer Law in conjunction with iterated data regression by PLS-1 or 2; check up on PLS-1 precision and correct computation;
- Examine the Calibration's Linear Correlations and Validation/ Composition Predictions

Computer Simulation Study of the PLS-1 Calibration Algorithm



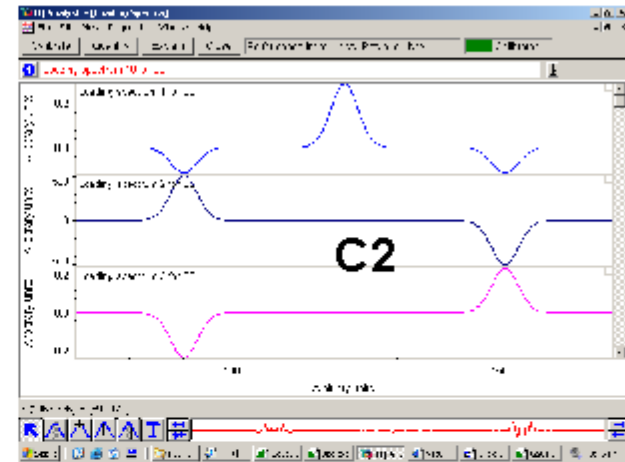
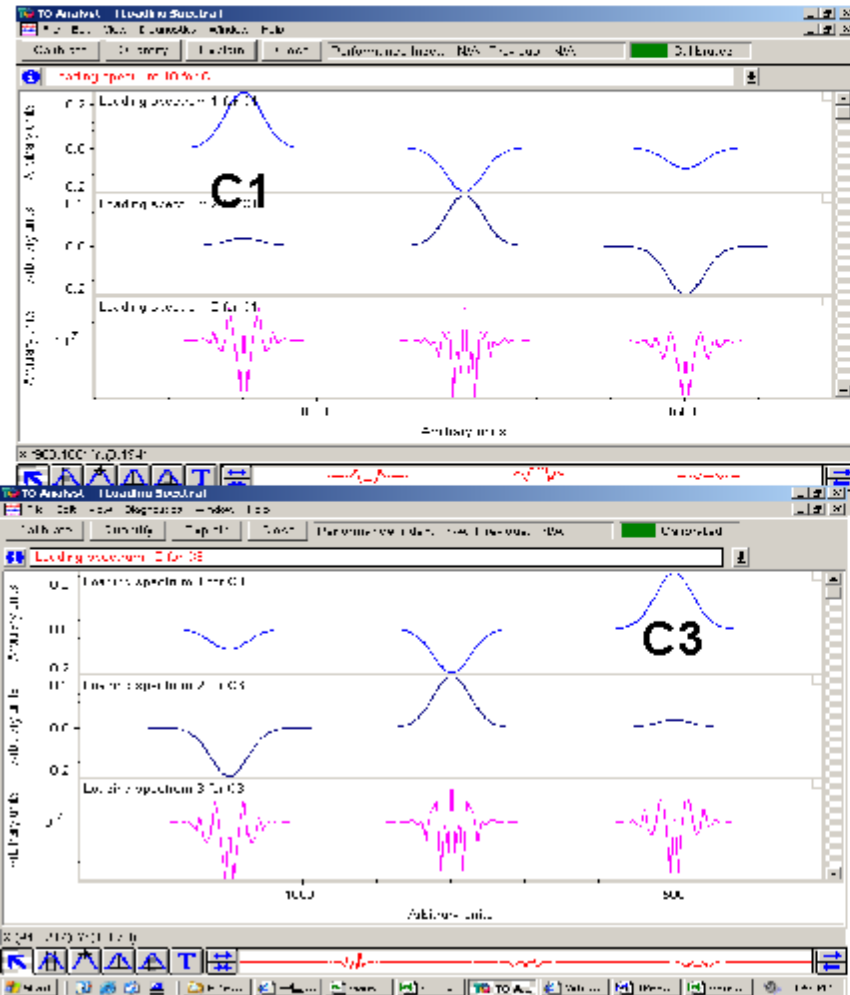
N=21, Calibration Standards

Computer Simulation of PLS-1 Algorithm with 3 Components (%)



Adapted from T. You, 2006

Loading Vectors and SECV' s of Components C1 to C3 for the Simulated PLS-1 Calibration Algorithm, without any Noise ...except from negligible PC computation errors !



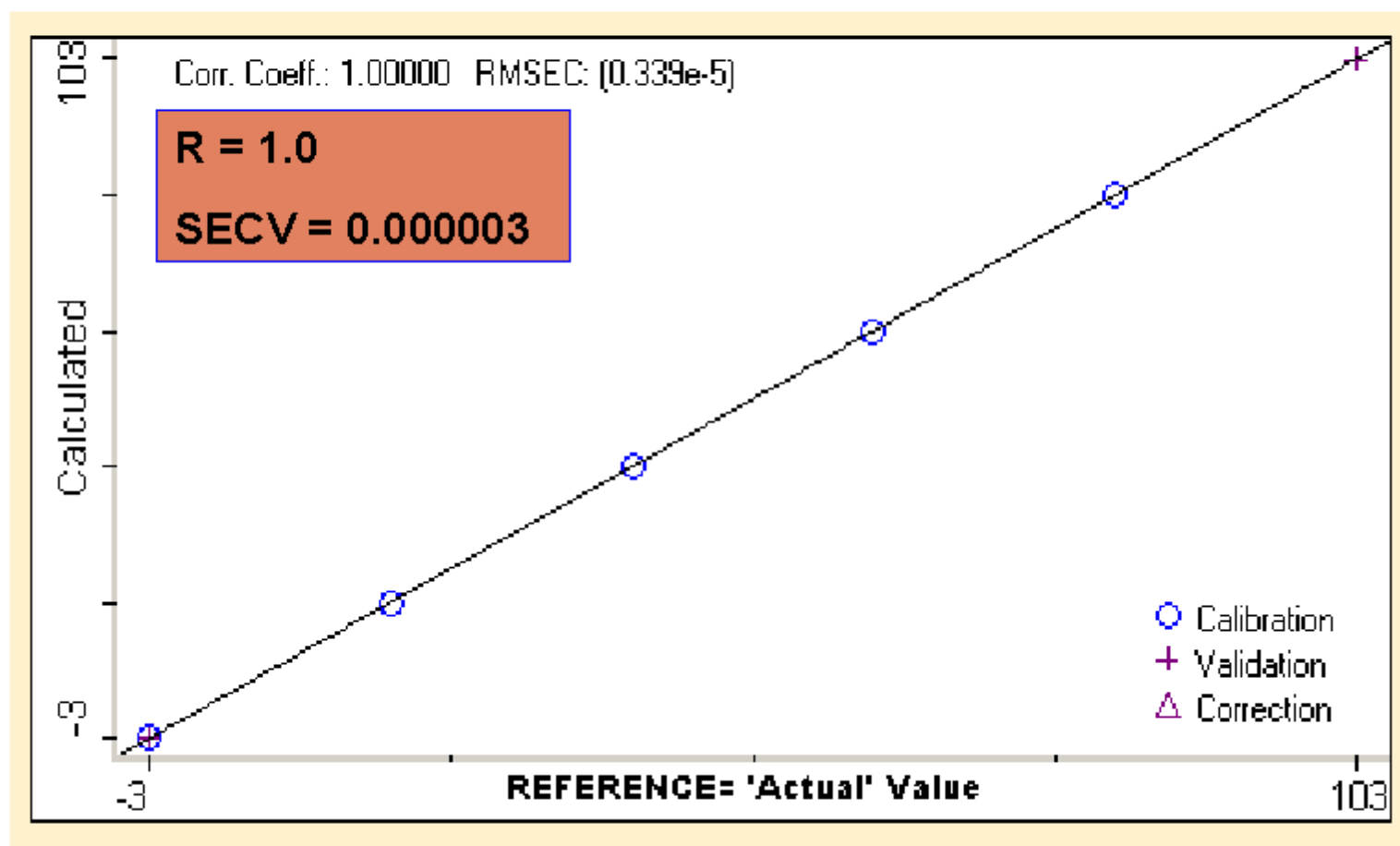
Component	Number of Factors	R	SEP
C1	2	100%	9×10^{-6}
C2	2	100%	1×10^{-6}
C3	2	100%	2×10^{-6}

R: Correlation coefficient

SEP: Standard Error of Prediction

* 'Ideal' conditions, that is, without Noise!

Predicted vs. Reference Concentrations of Component C1 for our PLS-1 Simulation Study



Source: T. You, 2006

II. Limitations and Advantages of Proposed Procedures

Major Limitations

- Amino Acids affected by acid hydrolysis of the protein:
 - Absence of Trp data. Trp is just an example of what hydrolysis does to amino acids. Corrections to hydrolysis is one of the limitations caused by hydrolysis
 - No data extrapolation to Zero
- Correlation of amino acids with Dry Protein
- Extremely limited range of ROV's makes it impossible to do the calculations for those amino acids

Matrix Effects

- A major obstacle that exists in the comparison of NIRS calibration data for different types of samples with the same chemical/biochemical composition but in different form or phase is the so-called “matrix effect.”
- This “matrix effect” depends on the state in which the molecules are in: Solid, Liquid phases and also on different: Texture/Morphology, Particle Size Distribution, Molecular Alignments.
- Other related causes : internal gaps, different interfaces, internal sample changes in refractive index, and so on.

NMR Advantages

- Trp data included
- *in situ* data acquisition

NIRS Calibrations for Selected AA Groups

- It is very important that there are enough knowns in the protein spectra to solve for all of the amino acid residues selected
- This research project will be using four groups of amino acid triplets such as:
 - Arg—Ala—Val
 - Cys (and/or Met)—Lys--Ser

Derivatized HPLC Advantages

- Derivatization prior to acid hydrolysis followed by HPLC eliminates the limitations caused by the protein treatment with acid.

Planned Work, p.2

Planned work also includes:

- Crystalline amino acids powders vs. amino acids in gels
- Egg white vs. egg albumin data
- The making of concentrated solutions, followed by partial drying resulting in an amorphous gel rather than crystalline powders.

Timeline and Steps

1. Select the best primary method for analyzing protein and amino acids
 - GCMS, NMR
 - Both are useable and comparable
 - NMR is superior
 - No acid hydrolysis
 - Readily available
1. Obtain dry protein and moisture contents
2. Obtain PLS-1 for protein content

Model Systems Rationale

- Why do we need a model system? Why can't we skip this step and just run our soybean samples?
- We need knowledge of the bands assigned from the model systems (e.g., Tyrosine ring, Amide II band)
- Serves to show the scale of the level of errors we can expect to get
- Others have tried and failed

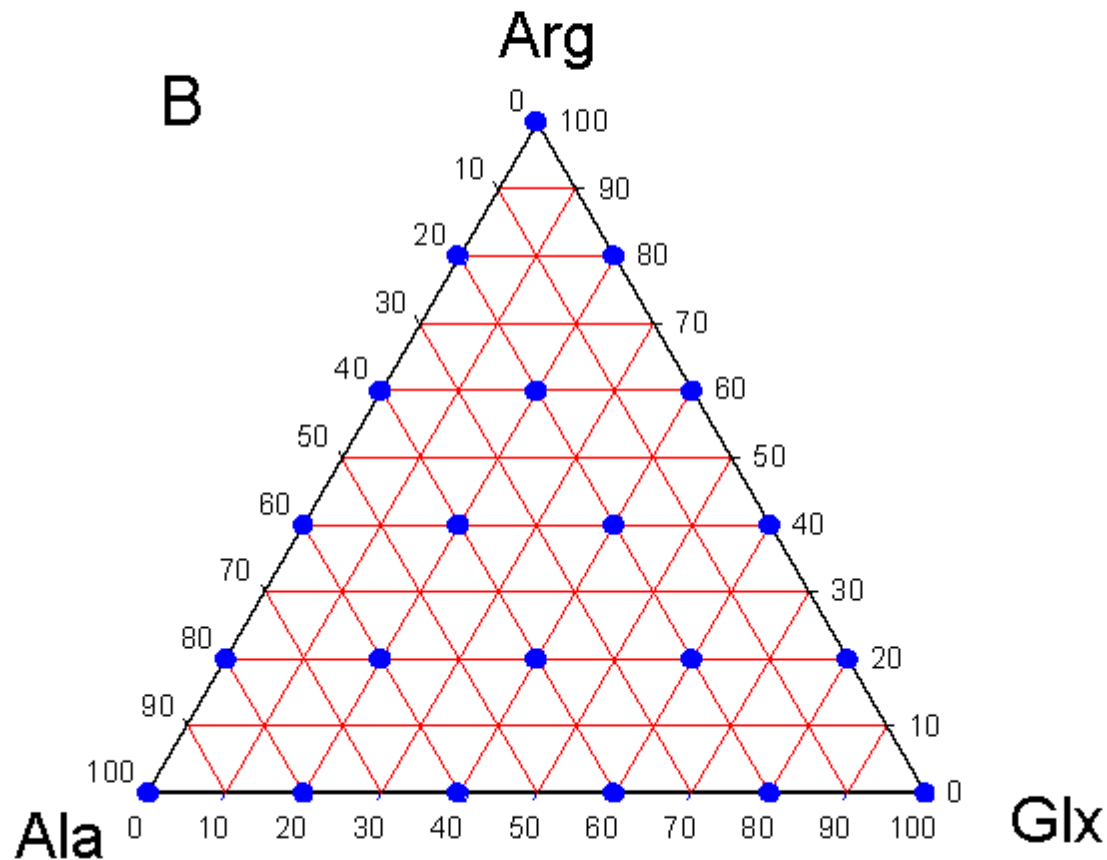
Timeline and Steps, cont'd

- In principle, all amino acid combinations should be performed, but we will do 4 triplets
- TIME FACTOR: Running the samples
 - 4 x 100 samples x 2 (duplicates)
 - = 800 samples
 - 5 minutes/sample.... 4,000 minutes
 - Or one month
- TIME FACTOR: Making the samples
 - 1 week per sample, parallel proc.
 - Total estimated time: several months

Experimental Setup

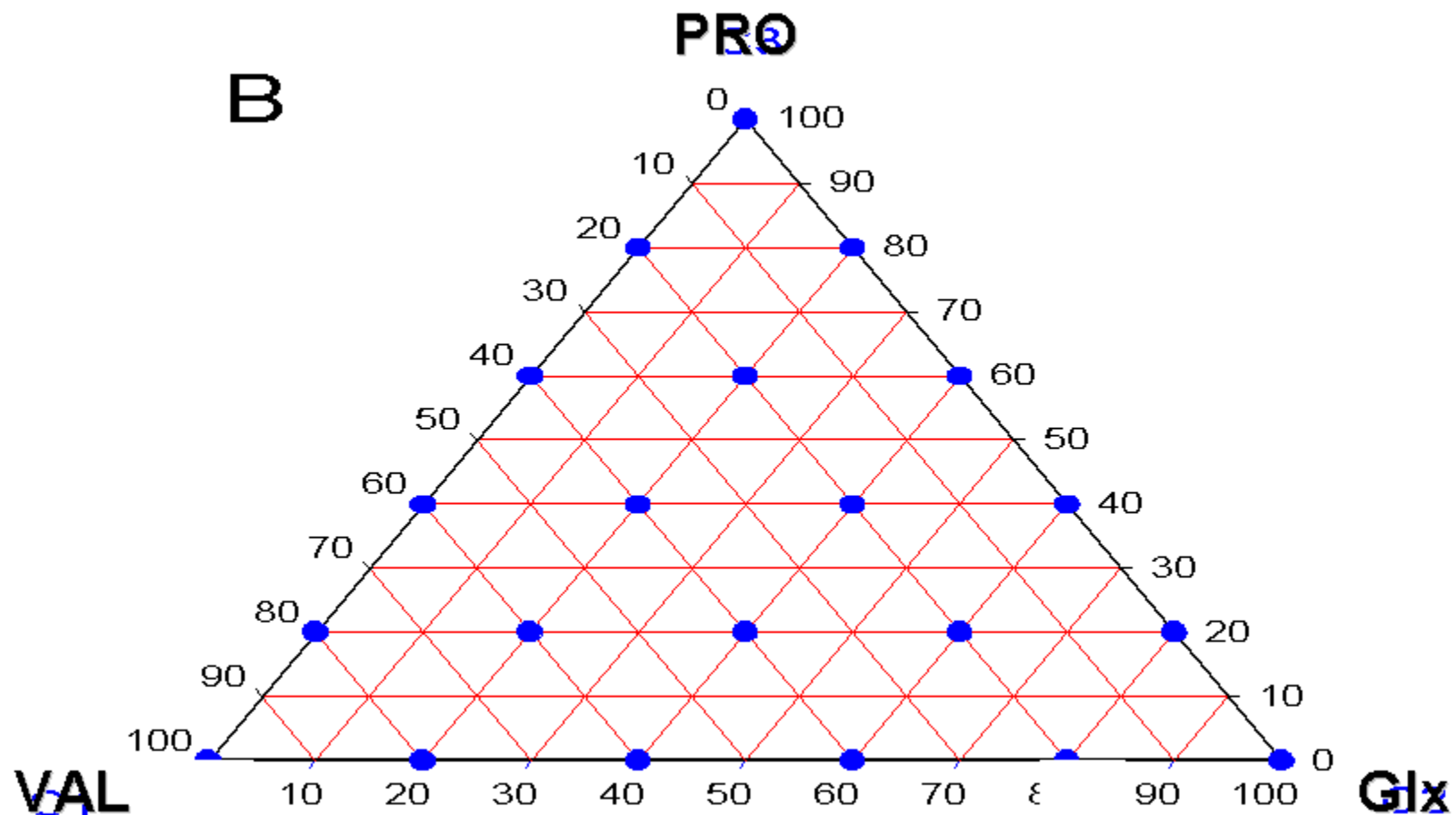
- For the selection of the individual amino acids to be analyzed, the following matrix is measured for pure amino acid powders:
- #1: Pro-Glu-Ala
- #2: Arg-pSer-Ile
- #3: Lys-Cys-Phe

Computer Simulation of PLS-1 Algorithm with 3 Components (%)



Adapted from T. You, 2006

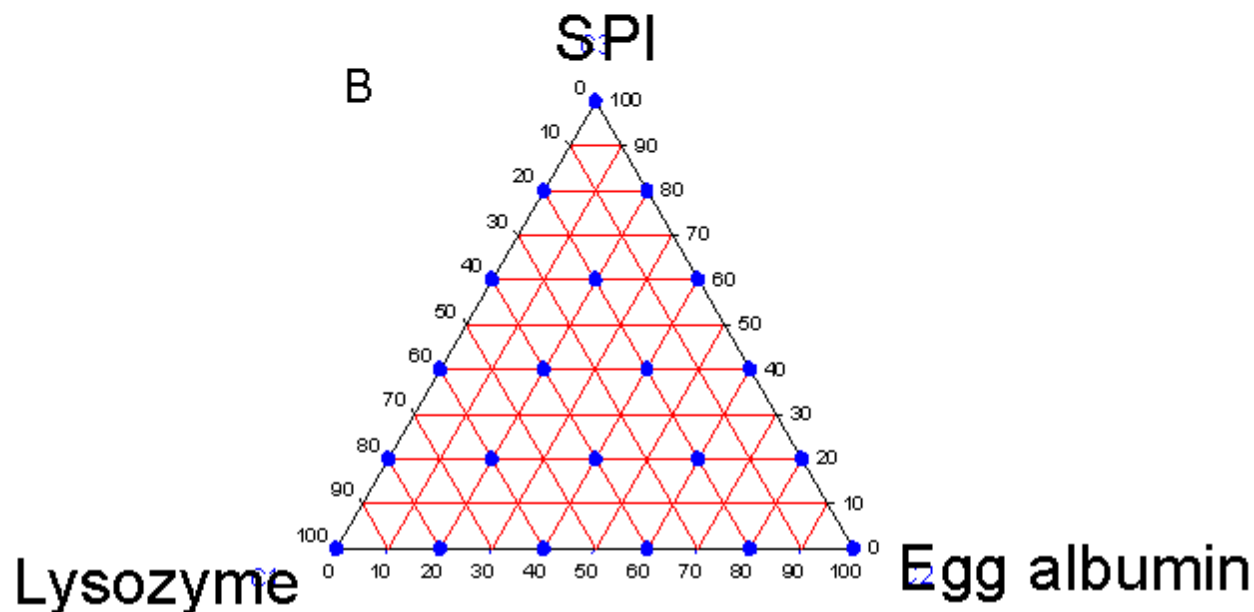
Planned Work, cont'd



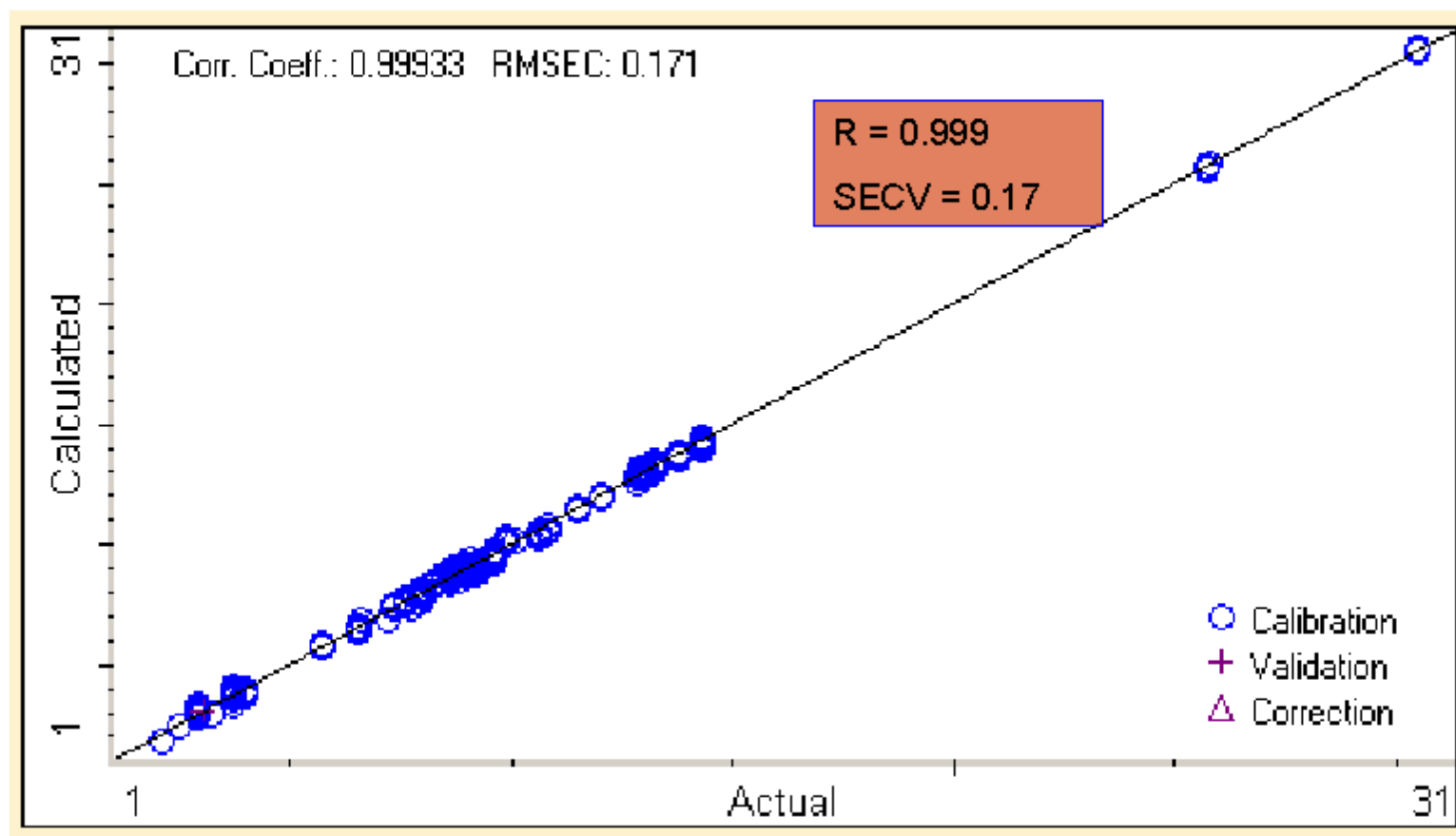
Similar model calibrations will be carried out for mixtures of other amino acids, triple selected, such as: Cys + Met - Arg-Ala or Met - Asn - Lys

Planned Work, cont'd

- Future work may include a “triangle” triplet of
 - Pro– Glx--Val
 - Met+Cys –Arg--Ala
 - Soy protein isolates (SPI), egg albumin and lysozyme



Moisture Calibration for Bulk Soybean Analysis on the Spectrum One NTS FT-NIR Instrument



65 calibration standards, 20 grams for each standard, 9mm NIR beam size

Source: Soybean NIR Database, UIUC

Bulk Soybean Calibration with 65 Standards for Protein, Oil, and Moisture Analysis

Developed with Data from the SpectrumOne NTS FT-NIR Spectrometer

Component	Number of Factors	R	SECV	SEP
Protein	13	99.9%	0.26	0.33
Oil	15	99.9%	0.13	0.23
Moisture	15	99.9%	0.17	0.30

R : Correlation Coefficient

SECV: Standard Error of Cross Validation

SEP: Standard Error of Prediction

Source: Soybean NIR Database, UIUC

Detrimental Effects of Light Scattering on the Accuracy of NIR Analysis for Whole Soybeans

(measured with the FT-NIR, SpectrumOne NTS Spectrometer)

	R		SECV	
Component	No MSC	MSC	No MSC	MSC
Protein	99.5	99.9	0.63	0.26
Oil	99.3	99.9	0.29	0.13
Moisture	99.8	99.9	0.26	0.17

R: Correlation coefficient

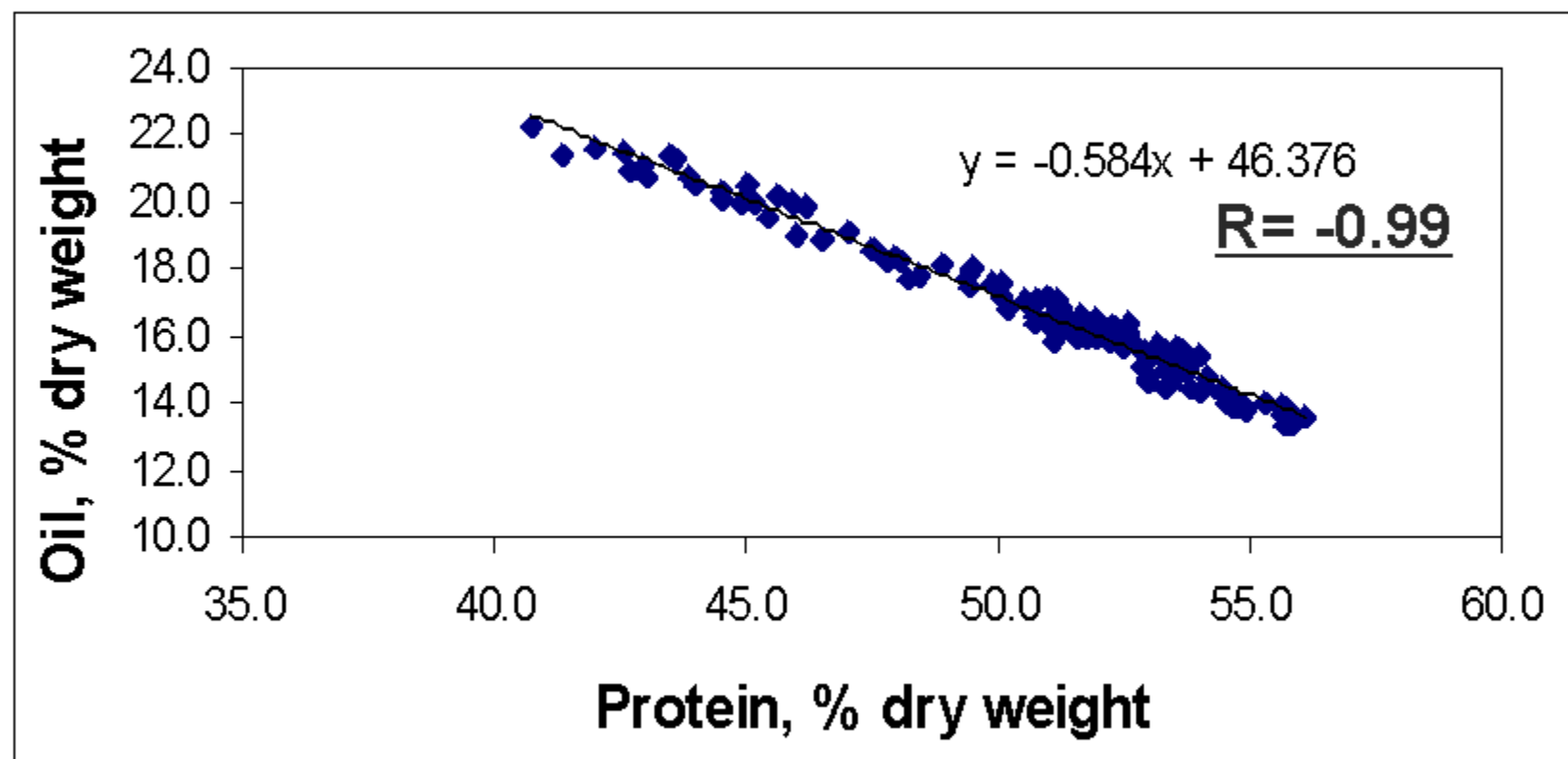
SECV: Standard Error of Cross Validation

(Tested with 65 bulk whole soybean standards)

Source: Soybean NIR Database, UIUC

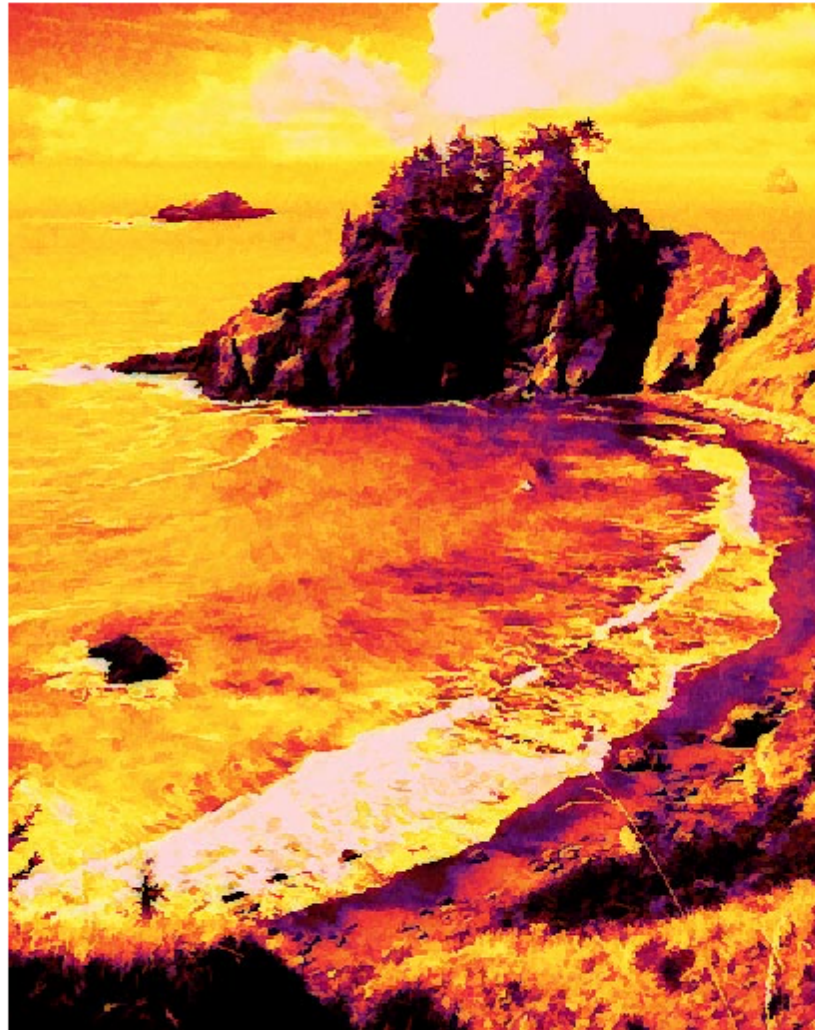
A new and Improved Set of 124 Bulk Soybean Standard Samples:

Protein-Oil Inverse Correlation for the Year 2003 Calibration Standard



* 124 Standard samples were selected with a wide range of Protein and Oil concentrations that were uniformly distributed in 0.5% concentration steps for Protein, and in 0.2% steps for Oil. Source: Soybean NIR Database, UIUC

NIR Illustration



Golden Cove (Digital Color Infrared)

Gold Beach, Oregon

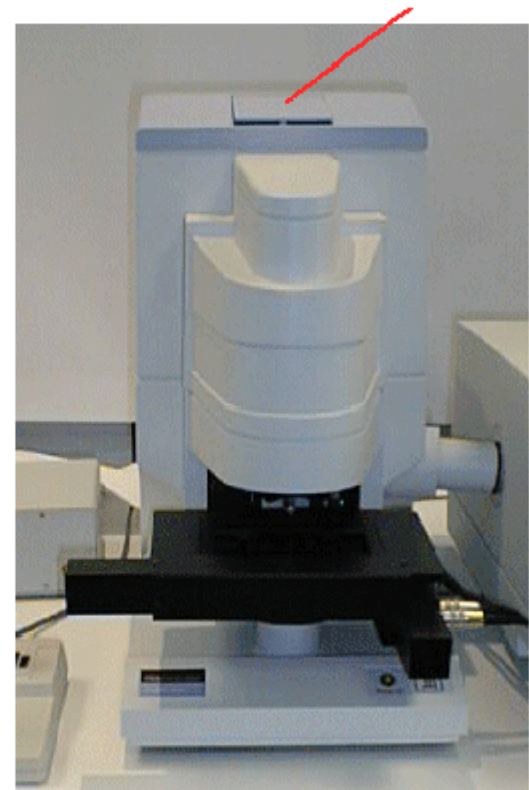
<http://www.pbase.com/image/38188240>

Chemical and Hyperspectral NIR Imaging

Chemical and Hyperspectral Imaging of Amino Acid Residues and Proteins in Soybeans

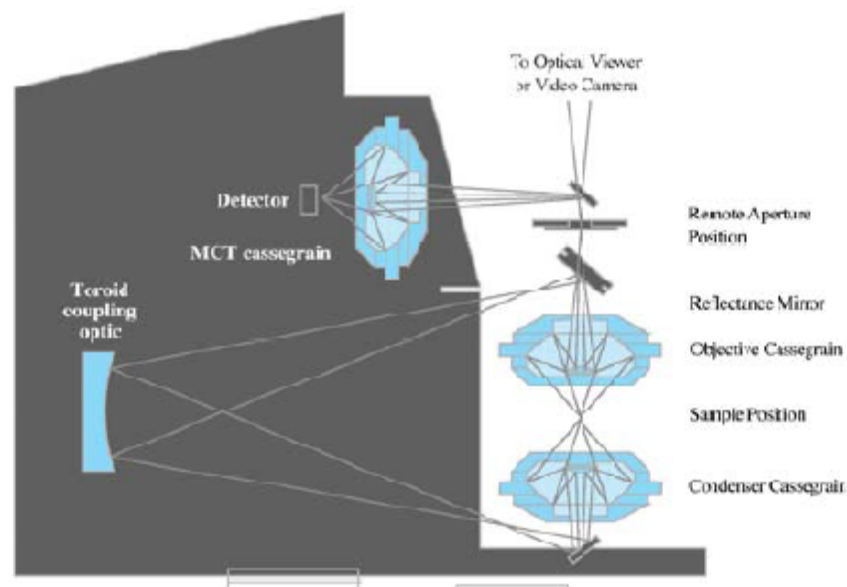
Images Using the NIR AutoImage FT-NIR Microspectrometer:

- Introduced in 2003 by PerkinElmer Co. (Shelton, CT, USA) for high-resolution studies.
- Employed for our NIR Microspectroscopy and Chemical Imaging investigations of Soybean seeds.

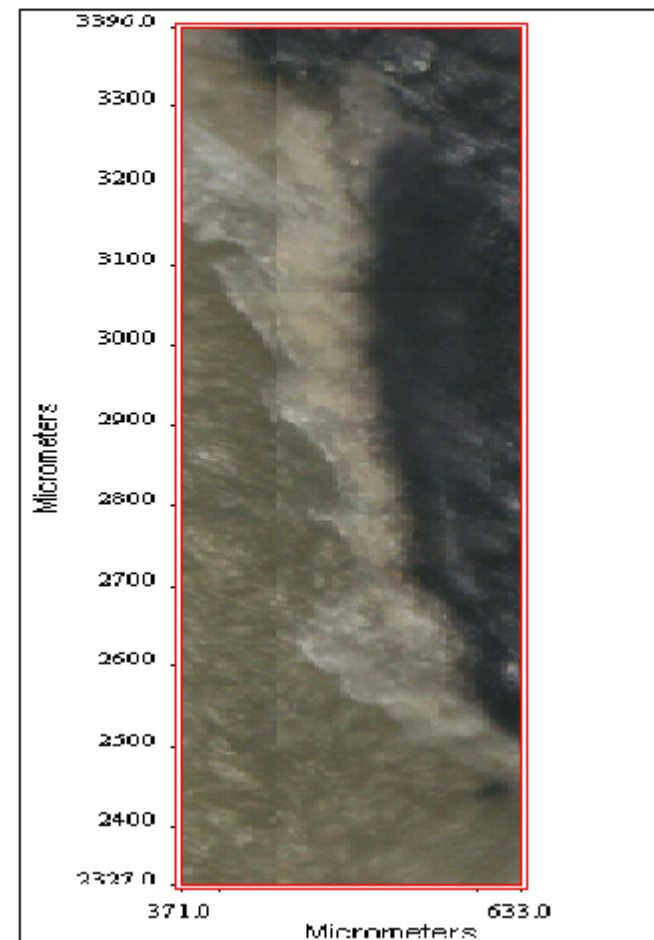
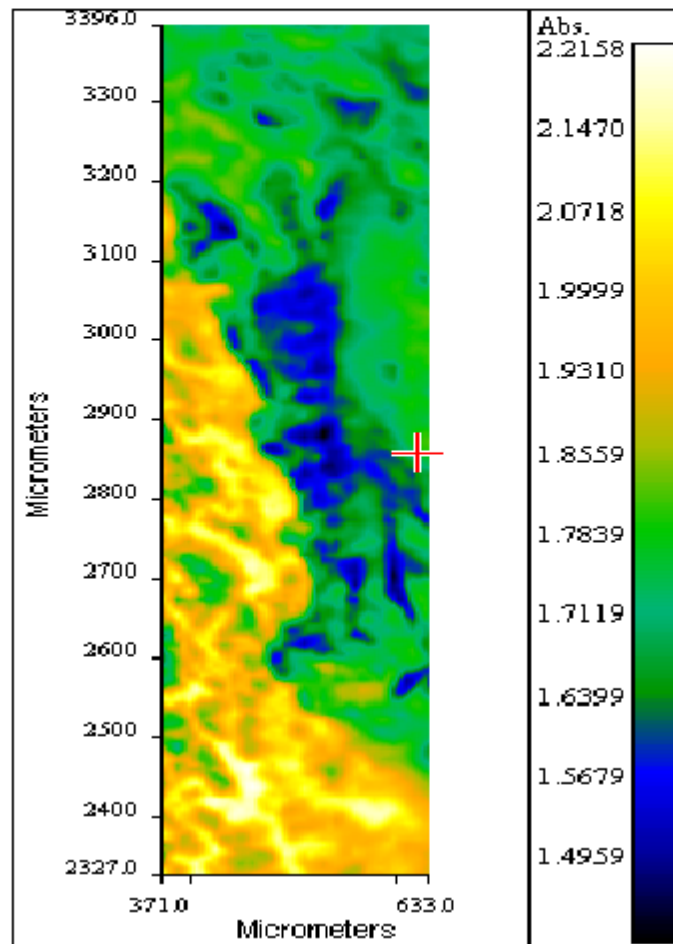


**Microscope Coupled to
the FT-NIR Spectrometer**

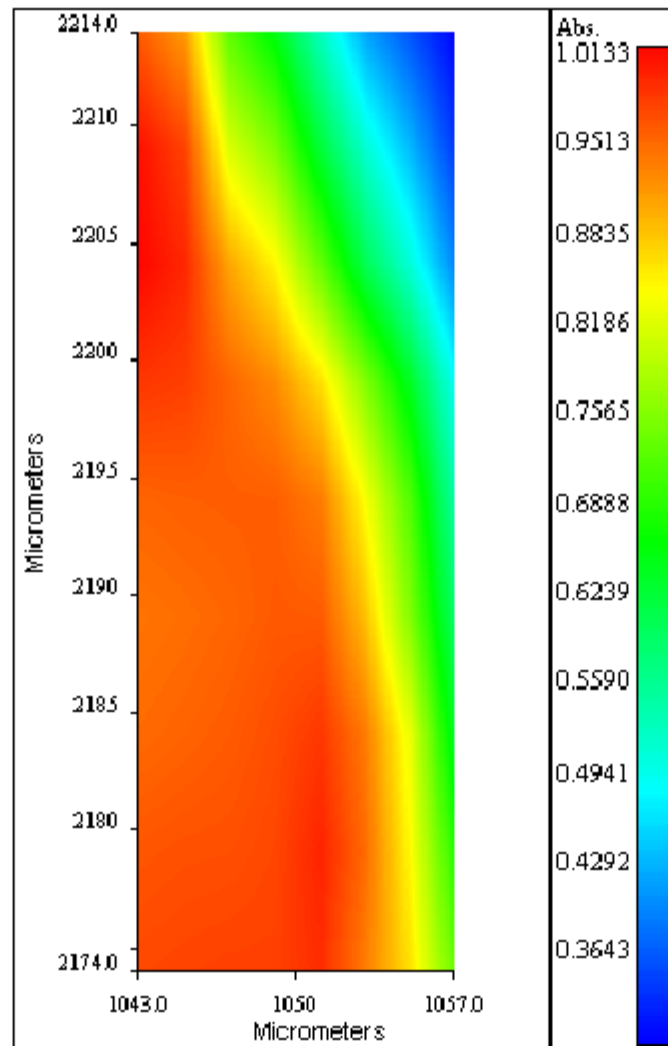
Image of the Perkin Elmer AutoImage Microspectrometer “Innards”



FT-IR Chemical Image (Left) and Visible Light Micrograph (Right) of a Black Coat Soybean with Part of the Coat Removed



FT-NIR Chemical Image of Oil Distribution in a Mature Soybean Embryo Section



Acknowledgements

- Renessen Biotech Corp
- Prof. Randall Nelson (Crop Sciences)
- Fmr. Research colleagues: Dr. Tiefeng You, Dr. Jun Guo
- Renée Prisecaru
- USDA Lab – fatty/amino acid analysis data
- Northern USDA Res.Ctr. for wet chemistry: GC-MS and HPLC aa data
- C-FAR
- ISPOB (Illinois Soybean Operating Board)

References

REFERENCES

- AACC. 1995. *Approved Methods of Analysis*, 9th Ed. The American Association of Cereal Chemists. St. Paul, Minnesota.
- AOAC International. 1995. *Official Methods of Analysis*, 16th ed., AOAC International, Gaithersburg, Maryland.
- AOCS. 1998. *Official Methods and Recommended Practices of the AOCS* – 5th Ed. The American Oil Chemists' Society. Champaign, Illinois.
- AOCS. 2002. Additions and Revisions to the Official Methods and Recommended Practices of the AOCS. AOCS Press. Champaign, IL. AK4-95.
- Augustine, M. and Baianu, I.C. 1984. High-Resolution ¹³C NMR and Ion Exchange Chromatography Amino Acid Profiling of Corn and Soybean Protein Hydrolysates. *FASEB Proceed.* 1125.
- Augustine, M.E., Baianu, I.C. 1986. High-Resolution ¹³C Nuclear Magnetic Resonance Studies of Maize Proteins. *J. Cereal Science.* 4: 371-378.
- Baianu, I.C. and H. Förster. 1980. Cross-Polarization, High-Field ¹³C NMR Techniques for Studying Physicochemical Properties of Wheat Grains and Wheat Proteins., *J. Applied Biochemistry*, 2: 347-355.
- Baianu, I.C. 1981. Carbon-13 and Proton NMR Studies of Wheat Proteins in Solution. *J. Sci. of Food & Agriculture*, 32: 309-313.
- Baianu, I.C., et al. 1990. Ch8. In *"NMR Applications in Biopolymers"*. Edited by J.W. Finley et al., Plenum Press, New York.
- Baianu, I.C. 1992. Ed. *"Physical Chemistry of Food Processes. Vol. 1: Fundamental Aspects."* Van Nostrand Reinhold. New York.
- Baianu, I.C., Pessen, H., Kumosinski, T.F. 1993. Ed. *"Physical Chemistry of Food Processes. Vol.2: Advanced Techniques, Structures, and Applications."* Van Nostrand Reinhold. New York.
- Baianu, I.C., You, T., Guo, J., Nelson, R. L. 2002. Calibration of Dual Diode Array and Fourier Transform Near Infrared Reflectance Spectrometers for Composition Analysis of Single Soybean Seeds in Genetic Selection, Cross-Breeding Experiments, Proceedings for the 9th Biennial Conference of the Cellular and Molecular Biology of the Soybean, August 11-14, 2002, 508.
- Baianu, I.C., Costescu, D., You, T. 2002. Novel Techniques for Microspectroscopy and Chemical Imaging Analysis of Soybean Seeds and Embryos, Proceedings for the 9th Biennial Conference of the Cellular and Molecular Biology of the Soybean, August 11-14, 2002, 509.
- Baianu, I.C., You T., Costescu, D.M., Lozano, P.R., Prisecaru, V. and Nelson, R.L. 2004a. Chapter 11 "High-Resolution Nuclear Magnetic Resonance and Near-Infrared Determination of Soybean Oil, Protein and Amino Acid Residues in Soybean Seeds", Luthria, D. Ed., *Oil Extraction and Analysis—Critical Issues and Comparative Studies*, AOCS Press. Champaign, IL. p193-240.
- Baianu, I.C., You T., Costescu, D.M., Lozano, P.R., Prisecaru, V. and Nelson, R.L. 2004b. Chapter 12 "Near-Infrared Microspectroscopy, Fluorescence Microspectroscopy, Infrared Chemical Imaging and High-Resolution Nuclear Magnetic Resonance Analysis of Soybean Seeds, Somatic Embryos and Single Cells, Luthria, D. Ed., *Oil Extraction and Analysis—Critical Issues and Comparative Studies*, AOCS Press. Champaign, IL. p241-273.
- Baianu, I.C., Korban, S.S., Costescu, D., You, T., Lozano, P., Hofmann, N.E. 2004c. Fourier Transform Near Infrared Microspectroscopy, Infrared Chemical Imaging, High-Resolution Nuclear Magnetic Resonance and Fluorescence Microspectroscopy Detection of Single Cancer Cells and Single Viral Particles. Cern. Ext-2004-069.
- K.A., and Lyman, C.M. 1948. Essential amino acid composition of soy bean meals prepared from twenty strains of soy beans. *J Biol. Chem.* 6: 29-36.

- Bardet, M., Foray, M.F., Bourguignon, J., and Krajewski, P. 2001. Investigation of Seeds with High-Resolution Solid-State ^{13}C NMR. *Magnetic Resonance in Chemistry*, 39: 733-738.
- Barton F.E. 2002. Theory and Principles of Near-Infrared Spectroscopy, *Spectroscopy Europe*, 14(1): 12-18.
- Belitz, H.D. and Grosch, W. 1987. *Food Chemistry*, Springer-Verlag, Berlin.
- Ben-Gera, I., Norris, K.H. 1968. Determination of Moisture Content in Soybeans by Direct Spectrophotometry. *Isr. J. Agric. Res.* 18: 125-132.
- Bardet, M., Foray, M.F., Bourguignon, J., and Krajewski, P. 2001. Investigation of Seeds with High-Resolution Solid-State ^{13}C NMR. *Magnetic Resonance in Chemistry*, 39: 733-738.
- Birth, G. S. 1986. The Light Scattering Characteristics of Ground Grains. *Intern. Agrophys.* 2(1): 59-67.
- Brim, C.A. and Burton, J.W. 1979. Recurrent Selection in Soybeans (*Glycine max*): 2. Selection for Increased Percent Protein in Seeds. *Crop Sci.* 19(4): 494-498.
- Burton, J.W., Wilson, R.F., Brim, C.A., and Rinne, R.W. 1989. Registration of Soybean Germplasm Line with Modified Fatty Acid Composition of Seed oil. *Crop Sci.* 29: 1583.
- Burton, J.W., and Wilson, R.F. 1994. Registration of N88-480, a Soybean Germplasm Line with High Concentration of Oil in Seeds. *Crop Sci.* 34: 313-314.
- Buning, P.H. and Diller, M. 2000. Rapid Analysis of Foods Using Near-Infrared Spectrometry (NIRS): Development, Use and New Possibilities. *Ernahrungs Umschau.* 47(1): 15-20.
- Buslov, D.K., Nikonenko, N.A., 1997. Regularized Method of Spectral Curve Deconvolution. *Applied Spectroscopy*, 51(5): 666-672.
- Buslov, D.K., Nikonenko, N.A., Sushko, N.I. and Zhbankov, R.G. 1999A. Analysis of the Results of α -D-glucose Fourier Transform Infrared Spectrum Deconvolution: Comparison with Experimental and Theoretical Data. *Spectrochimica Acta Part A.* 55: 229-238.
- Buslov, D.K., Nikonenko, N.A., Sushko, N.I. and Zhbankov, R.G. 1999B. Deconvolution of Fourier Transform Infrared Spectrum of α -D-Galactose: Comparison with Experimental and Theoretical Data. *Spectrochimica Acta Part A.* 55: 1101-1108.
- Cameron, A.G. 1967. An Assessment of the Potential Application of the Method of Attenuated Total Reflectance (ATR) Infrared Qualitative Analysis to Food Materials. *J. Food Technology.* 2:223.
- Carrao, P.M., de-Goes, F.S. and Kikuchi, A. 2002. Extraction Time for Soybean Isoflavone Determination. *Brazilian Archives Bio. Technol.* 45 (4): 515-518.
- Carr, H.Y., and Purcell, E.M. 1954. Effects on Diffusion on Free Precession in Nuclear Magnetic Resonance Experiments. *Physical Review*, 94 (3): 630-638.
- Cinier, R., Cuilment, J. 1998. PLS Calibration for Precise and Practical Quantitative Analysis of Simple Solutions Using NIR Spectroscopy. *Bruker Report*, 146:6-9.
- Coblentz, W.K. 1982. A Study in the Near Infrared Reflectance Spectra of Proteins and Related Compounds. MS thesis. Pennsylvania State University.
- Costescu, D.M. 2003. MS Thesis, University of Illinois at Urbana-Champaign, Urbana, Illinois.

- Delwiche, S.R., 1995. Single Wheat Kernel Analysis by Near-Infrared Transmittance: Protein Content. *Cereal Chem.* 72: 11-16.
- Derome, A.E. 1987. *Modern NMR Techniques for Chemistry Research*, p. 129, Pergamon Press, New York.
- Dev, S.B., Rha, C.K. and Walder, F. 1984. Secondary Structural Changes in Globular Protein Induced by a Surfactant: Fourier Self-Deconvolution of FT-IR Spectra. *J. Biomolecular Structure & Dynamics*. 2(2): 431-442.
- Diaspro, A., and Robello, M. 1999. Multi-Photon Excitation Microscopy to Study Biosystems. *European Microscopy and Analysis*, 5: 5-7.
- Diem, M. 1993. Ed. *Introduction to Modern Vibrational Spectroscopy*. John Wiley & Sons. New York, NY.
- Doty, D. M., Bergdoll, M. S., Nash, I. A., and Brunson, A. M. 1946. *Cereal Chem.* 23:199.
- Eigen, M. and Rigler, R. 1994. *Proc Natl. Acad. Sci. USA*. **91**: 5740.
- Fahrenfort, J. 1961. Attenuated Total Reflection. A New Principle for the Production of Useful Infrared Reflection Spectra of Organic Compounds. *Spectrochim. Acta* 17: 698
- Feynman, R.P. 1963. *"Lectures on Physics", Vol.3*, Addison-Wesley Publishing Company, Reading, MA.
- Fraenkel-Conrat, H, Cooper, M., 1944. The Use of Dyes for the Determination of Acid and Basic Groups in Proteins, *J. Biol. Chem.*, 154: 239-246.
- Galactic Industries Corporation. 1996. GRAMS/32 User's Guide: PLS plus/IQ for GRAMS/32 and GRAMS/386, Chapters 1-4.
- Geladi, P., MacDougall, D., Martens, H. 1985. Linearization and Scatter-Correction for Near- Infrared Reflectance Spectra of Meat. *Applied Spectroscopy*. 39(3): 491-500.
- Goldflus, F., Ceccantini, M., and Santos, W. Amino Acid Content of Soybean Samples Collected In Different Brazilian States: Harvest 2003/2004. *Rev. Bras. Cienc. Avic.* [Online]. 2006, 8(2): 105-111.
- Guo, J., You T., Baianu, I.C., Nelson, R.L. 2002. Evaluation of Four Dispersive NIR Instruments and Methodology Development for Soybean Composition Analysis in Genetic Selection and Breeding Programs, Proceedings of the China and International Soy Conference and Exhibition 2002 (CISCE 2002), November 6-9, 2002, 412-413.
- Guo, J. 2004. Development of Dispersive and Fourier Transform Near Infrared Spectroscopy Methodology for Food and Edible Seed Analysis. PhD Thesis, University of Illinois at Urbana-Champaign, Urbana, Illinois.

- Kuiken, Klempir J. M. 1999. Nuclear Magnetic Resonance Characterization of Soybeans for Oil Content Measurement. MS Thesis, University of Illinois at Urbana-Champaign, Urbana, Illinois.
- Haaland, D.M., Thomas, E.V. 1988. Partial Least-Squares Methods for Spectral Analyses. Relation to Other Quantitative Calibration Methods and the Extraction of Qualitative Information. *Analytical Chemistry*, 60: 1193-1202.
- Hammond, E.G., and Fehr, W.R. 1982. Progress in Breeding for Soybean Oil with Low Linolenic Acid. *J. Am. Oil Chem. Soc.* 59 (4): 304A.
- Hammond, E.G., and Fehr, W.R. 1983. Registration of A6 Germplasm Line of Soybean. *Crop Sci.* 23: 192-193.
- Harrick, N. J. 1960. Surface Chemistry from Spectral Analysis of Totally Internal Reflected Radiation. *J. Phys. Chem.* 64:1110.
- Haswell, S.J. 1992. *Practical Guide to Chemometrics*. Marcel Dekker, Inc., New York.
- Harris, Robin K. 1983. *Nuclear Magnetic Resonance spectroscopy, A Physicochemical View*, Pitman, London.
- Howard Mark. 2001. *Near-Infrared Applications in Biotechnology, (Biochemical and Pharmaceutical)*. Chapter 11 "Fundamentals of Near-Infrared Spectroscopy", P. 312, Edited by Raghavachari, R. Promega Corporation, Madison, Wisconsin.
- Hymowitz, T., Collins, F.I., Panczner, J. and Walker, W.M. 1972. Relationship between the Content of Oil, Protein, and Sugar in Soybean Seed. *Agron. J.* 64: 613-616.
- Isaksson, T., Miller, C.E. and Naes, T. 1992. Nondestructive NIR and NIT Determination of Protein, Fat and Water in Plastic Wrapped, Homogenized Meats. *Applied Spectroscopy*. 46: 1685.
- Isaksson, T., Naes, T. 1988. The Effect of Multiplicative Scatter Correction (MSC) and Linearity Improvement in NIR Spectroscopy. *Applied Spectroscopy*. 42(7): 1273-1284.
- Jones, D.B. 1931. Factors for Converting Percentages of Nitrogen in Foods and Feeds into Percentages of Nitrogen , USDA Circular No. 183, Washington DC.
- Joslyn, M.A. 1970. *Methods in Food Analysis*, 2nd ed. Academic Press, New York.
- Kabelka, E.A., Diers, B.W., Fehr, W.R., LeRoy, A.R., Baianu, I.C., You, T., Neece, D. J., Nelson, R. L. 2004. Putative Alleles for Increased Yield from Soybean Plant Introductions. *Crop Sci.*, 44:784-791.
- Kakalis, L.T. and Baianu, I.C. 1989. A High-Resolution ¹³C NMR Study of Glycinin in D₂O Solutions. *J. of Agric. & Food Chem.* 37: 1222-1228.
- Kakalis, F.T. and Baianu, I.C. 1990. High-Resolution ¹³C NMR Study of the Soybean 7S Storage Protein Fraction in Solution. *J. of Agric. & Food Chem.* 38: 1314-1320.
- Kettling, U., Koltermann, A., Schwille, P., and Eigen, M. 1998. Real-Time Enzyme Kinetics Monitored by Dual-Color Fluorescence Cross-Correlation Spectroscopy. *Proc. Natl. Acad. Sci. USA* 95: 1416-1420.
- Klempir J. M. 1999. Nuclear Magnetic Resonance Characterization of Soybeans for Oil Content Measurement. MS Thesis, University of Illinois at Urbana-Champaign, Urbana, Illinois.
- Kortum, G. 1969. *Reflectance Spectroscopy--Principles, Methods, Applications*. Springer-Verlag, NewYork, NY.

- Lamb, D., Hurburgh, C.R. 1991. Moisture Determination in Single Soybean Seeds by Near-Infrared Transmittance. *Trans. ASAE*. 34: 2123-2129.
- Lee, S.J., Ahn, J.K., Kim, S.H., Kim, J.T., Han, S.J., Jung, M.Y. and Chung, I.M. 2003. Variation in Isoflavone of Soybean Cultivars with Location and Storage Duration. *J. Agric. Food Chem.* 51(11): 3382-3389.
- Liu, K.S. 1997. *Soybeans: Chemistry, Technology, and Utilization*. Chapman & Hall, New York, NY. 532p.
- Liu, K.S. 2000. Expanding Soybean Food Utilization. *Food Technology*. 54(7): 46-58.
- Lowry, O. H., N.J. Rosebrough, A. L. Farr and R. J. Randall, 1951. Protein Measurement with the Folin Phenol Reagent. *J Biol. Chem.* 193: 265-275.
- Madden, H.M., 1978. Comments on the Savitzky-Golay Convolution Method for Least-Squares Fit Smoothing and Differentiation of Digital Data. *Anal. Chem.* 50: 1383.
- Maluszynski, M., Ahloowalia, B.S. and Sigurbjornsson, B. 1995. Application of *in vivo* and *in vitro* Mutation Techniques for Crop Improvement. *Euphytica*, 85: 303-315.
- Mansur, L.M., Orf, J.H., Chase, K., Jarvik, T., Cregan, P.B. and Lark, K.G. 1996. Genetic Mapping of Agronomic Traits Using Recombinant Inbred Lines of Soybean. *Crop Sci.* 36: 1327-1336.
- Mark, H. 2001. *Near-Infrared Applications in Biotechnology*, Chapter 11 "Fundamentals of Near Infrared Spectroscopy", p.312, Editor Raghavachari, R. Promega Corporation, Madison, Wisconsin.
- Martens, H., Jensen, S.A. and Geladi, P. 1983. Proceedings, Nordic Symposium on Applied Statistics, Stavanger, June 1983, pp.205-234.
- Mie, G. 1908. *Ann. Physik.* 4: 25, 377.
- Minson, D.J., Tuckett, P.G, Law D.P. 1985. A Comparison of three Methods of Increasing the Precision of Regression Used for Estimating Nutritive Value of Forages by Near Infrared Reflectance. 1985, NIR84 Proc. Int. Symp. Near Infrared Reflectance Spectroscopy., 1st. RACI, Cereal Chem. Div. Melbourne, Australia. pp 138-148.
- Myoung, G.C., In, Y.B., Sung, T.K., Won, Y.H., Doo, C.S., Huhn, P.M. and Kwang, H.K. 2001A. Determination of Protein and Oil Contents in Soybean Seed by Near Infrared Reflectance Spectroscopy. *Korean J Crop Sci.* 46(2): 106-111.
- Myoung, G.C., In, Y.B., Sung, T.K., Won, Y.H., Doo, C.S., Huhn, P.M. and Kwang, H.K. 2001B. Non-Destructive Method for Selection of Soybean Lines Contained High Protein and Oil by Near Infrared Reflectance Spectroscopy. *Korean J Crop Sci*, 46(5): 401-406.

- Nieh C.D. and Snyder, H.E. 1991. Solvent Extraction of Oil from Soybean Flour: I. Extraction Rate, a Countercurrent Extraction System, and Oil Quality. *J. Am. Oil Chem. Soc.* 68(4): 246-249.
- Nielsen, S.S. 1994. *Introduction to the Chemical Analysis of Foods*. Jones and Bartlet Publishers, Boston, London, Chapters 7, 10, 14.
- Norris, K.H. and Williams, P.C. 1984. Optimization of Mathematical Treatment of Raw Near-IR Signal in the Measurement of Protein in Hard Red Spring Wheat: 1. Influence of Particle Size. *Cereal Chem.* 62: 158-165.
- Norris, K.H. 1983. Using Gap Derivatives as Pre-processing for Quantitative Models, Food Research & Data Analysis, Proceedings of the 1982 IUFST Symposium, Martens, H. Ed., Applied Science Publishers, Oslo, 46-47.
- Ohnishi, S.T. and Barr, J.K. 1978. A Simplified Method of Quantitating Protein Using the Biuret and Phenol Reagents. *Anal. Biochem.* 86: 193-200.
- Openshaw, S.J. and Hadley, H.H. 1984. Selection Indexes to Modify Protein Concentration of Soybean Seeds. *Crop Sci.* 24: 1-4.
- Orman, B.A., Schumann, R.A., 1992. Nondestructive Single-Kernel Oil Determination of Maize by Near-Infrared Transmission Spectroscopy. *J. Am. Oil Chem. Soc.* 69: 1036-1038.
- Osborne, B. G., Fearn, T. and Hindle, P.H. 1993. *Practical NIR Spectroscopy with Applications in Food and Beverage Analysis*, 2nd Edition. pp 13-21, 99-117. Longman Scientific & Technical, Harlow, UK. 227p.
- Paulsen, M.R., Bajaj, M. 2000. Using Unscrambler for Extractable Corn Starch Calibration. ASAE Paper No. 00-3091. ASAE. St. Joseph, MI.
- PerkinElmer Instruments, 2000. Spectrum One User's Guide, Shelton, CT 06484, USA.
- PerkinElmer Instruments, 2002. AutoImage User's Guide, Sheldon, CT 06484, USA.
- Peterson, G. L. 1979. Review of the Folin Phenol Protein Quantitation Method of Lowry, Rosebrough, Farr and Randall. *Analytical Biochemistry* 100: 201-220.
- Pfeffer, P. and Gerasimowicz, W. (Eds.). 1987. *NMR in Agriculture*. CRS Publishers, Boca Raton, FL.
- Pomeranz Y. and Meloan, C.E. 1994. *Food Analysis Theory and Practice*, 3rd edition, chapter 33, 567-574. Chapman & Hall, New York, NY.

- Raghavachari, R. 2001. *Near-Infrared Applications in Biotechnology*, Marcel-Dekker, New York, NY.
- Reffer, J.A., Martoglio, P.A. 1995. *Uniting Microscopy and Spectroscopy, Practical Guide to Infrared Microspectroscopy*, Chapter 2, p.41, Edited by Howard J. Humecki, Marcel Dekker, New York, NY.
- Rigler R. and Widengren J. 1990. Ultrasensitive Detection of Single Molecules by Fluorescence Correlation Spectroscopy, *BioScience* (Ed. Klinge & Owman) p.180.
- Rippe, K. 2000. Simultaneous Binding of Two DNA Duplexes to the NtrC-Enhancer Complex Studied by Two-Color Fluorescence Cross-Correlation Spectroscopy. *Biochemistry* 39(9): 2131-2139.
- Robertson, J. A. and F. E. Barton.1984. Oil and Water Analysis of Sunflower Seeds by Near Infrared Reflectance spectroscopy, *JAOCS*, 61 (3): 543-547.
- Robinson, H.W. and C.G. Hodgen 1940. The Biuret Reaction in the Determination of Serum Protein. 1. A Study of the Conditions Necessary for the Production of the Stable Color which Bears a Quantitative Relationship to the Protein Concentration. *Journal of Biological Chemistry* 135: 707-725.
- Rutar, V. 1982. A New Possibility for Nondestructive Protein Content Determination in Viable Seeds, *Applied Spectroscopy*, 36: 259-260.
- Rutar, V., M. Kovac and G. Lahajnar.1989. Nondestructive Study of Liquids in Single Seeds Using Nuclear Magnetic Resonance and Magic Angle Sample Spinning, *JAOCS*, 66: 961-965.
- Rutar, V. 1984. NMR Studies in Intact Seeds, in *Nuclear Magnetic Resonance in Agriculture*. editors Philip E. Pfeffer and Walter V. Gerasimowicz, CRC Press Inc., Boca Raton, Florida.
- Rutar, V.1989. Magic Angle Spinning NMR Spectroscopy of Liquids as Nondestructive Method for Studies of Plant Seeds, *J. Agric. Food Chem.*, 37: 67-70.

- Savitsky, A. and Golay, M.J. 1964. Smoothing and Differentiation of Data by Simplified Least Squares Procedures. Anal. Chem. 36: 1627-1639.
- Schwille P., Oehlenschläger F. and Walter N. 1996. Analysis of RNA-DNA Hybridization Kinetics by Fluorescence Correlation Spectroscopy, Biochemistry 35: 10182.
- Schwille, P., Bieschke, J. and Oehlenschläger F. 1997. Kinetic Investigations by Fluorescence Correlation Spectroscopy: The Analytical and Diagnostic Potential of Diffusion Studies, Biophys. Chem. 66: 211-228.
- Schwille P., Meyer-Almes F.-J., and Rigler R. 1997. Dual-Color Fluorescence Cross-Correlation Spectroscopy for Multicomponent Diffusional Analysis in Solution, Biophys. J. 72: 1878-1886.
- Schwille, P., Haupts, U., Maiti, S., and Webb. W. 1999. Molecular Dynamics in Living Cells Observed by Fluorescence Correlation Spectroscopy with One- and Two-Photon Excitation. Biophysical Journal, 77(10):2251-2265.
- Schwille, P. 2001. *Fluorescence Correlation Spectroscopy. Theory and applications*. Rigler R., and Elson, E. S. eds. Springer Verlag: Berlin. p360.
- Shchegolikhin, A., Lazareva, O. 2000. Diffuse Reflectance for the Routine Analysis of Liquids and Solids. Internet Journal of Vibration Spectroscopy [www.ijvs.com]. 4(1): 8-38.
- Shadow, W. 1998. *Rapid Analysis for the Food Industry Using Near-Infrared Spectroscopy*. Perten Instruments North America, Inc. 17p.
- Shadow, W. and A. Carrasco. 2000. Practical Single-Kernel NIR/Visible Analysis for Small Grains. Cereal Foods World 45(1): 16-18.
- Silvela, L., Rodgers, R., Barrera, A., Alexander, D.E. 1989. Effect of Selection Intensity and Population Size on Percent Oil in Maize, Zea mays L. Theoretical and Applied Genetics 78: 298-304.
- Simpson, A.M. Jr. and Wilcox, J.R. 1983. Genetic and Phenotypic Associations of Agronomic Characteristics in Four High Protein Soybean Populations [Glycine max]. Crop Sci. 23: 1077-1081.

- Smith, A.L. 1979. Ed. *Applied Infrared Spectroscopy: Fundamentals, Techniques, and Analytical Problem-solving*. John Wiley & Sons. New York, NY.
- Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J., and Klenk, D.C. 1985. Measurement of Protein Using Bicinchoninic Acid. *Anal. Biochem.* 150: 76-85.
- Song, T., Barua, K., Buseman, G. and Murphy, P.A. 1998. Soy Isoflavone Analysis: Quality Control and a New Internal Standard. *Am. J. Clinical Nutrition.* 68(6), Supplement, 1474S-1479S.
- Sorvaniemi, J., Kinnunen, A., Tsados, A., Malkki, Y. 1993. Using Partial Least Squares Regression and Multiplicative Scatter Correction for FT-NIR Data Evaluation of Wheat Flours, *Lebensmittel-Wissenschaft & Technologie*, 26(3): 251-258.
- Specht, J., Nelson, R.L., Webster, R., Naidu, S., Ainsworth, E. and Ort, D. 2002. A Genomic Perspective on the Soybean Protein(+)/Oil(-)/Yield(-) Enigma. Program and Proceedings for the 9th Biennial Conference of the Cellular and Molecular Biology of the Soybean, August 11-14, 2002, P.202.
- Stuber, C.W. 1992, Biochemical and Molecular Markers in Plant Breeding. *Plant Breeding Rev.* 9: 37-61.
- Tajuddin, T., Watanabe, S., Masuda, R., Harada, K. and Kawano, S. 2002. Application of Near Infrared Transmittance Spectroscopy to the Estimation of Protein and Lipid Contents in Single Seeds of Soybean Recombinant Inbred Lines for Quantitative Trait Loci Analysis. *J. Near Infrared Spectrosc.* 10(4): 315-325.
- Tekel, J., Daeseleire, E., Heeremans, A. and Peteghem, C.V. 1999. Development of a Simple Method for the Determination of Genistein, Daidzein, Biochanin A, and Formononetin (Biochanin B) in Human Urine. *J. Agric. Food Chem.* 47(9): 3489-3494.
- Theissing, H. H. 1950. *J. Opt. Soc. Am.* 40: 232.
- Thompson, J.A. and Nelson, R.L. 1998. Utilization of Diverse Germplasm for Improving Soybean Yield. *Crop Sci.* 38: 1362-1368.
- Thompson N.L. 1991. In *Topics of Fluorescence Spectroscopy*. Lakowicz, J.R. ed. Plenum Press, Vol 1 p 337.
- Tiwari, P. N. and Gambhir, P. N. 1995. Seed Oil Determination without Weighing and Drying the Seeds by Combined Free Induction Decay and Spin-Echo Nuclear Magnetic Resonance Signals, *JAOCS*, 72(9).
- USDA National Agriculture Statistical Service (NASS). 1999. USDA-NASS Agricultural Statistics 1999. <http://www.usda.gov/nass/pubs/agr99/acro99.htm>.
- Velasco, L., Goffman, F.D., and Becker H.C. 1999. Development of Calibration Equations to Predict Oil Content and Fatty Acid Composition in Brassicaceae Germplasm by Near Infrared Reflectance Spectroscopy. *JAOCS*, 76: 25-30.
- Verna, D.P.S., Shoemaker, R.C. 1996. *Soybean Genetics, Molecular Biology and Biotechnology*. Cab International. Guildford, UK.

- Watson et al. 1987. Ed. *Corn: Chemistry and Technology*. American Association of Cereal Chemists. Inc. St. Paul, MN.
- Wang, D., Dowell, F.E., Lacey, R.E. 1999. Single Wheat Kernel Color Classification Using Near-Infrared Reflectance Spectra. *Cereal Chem.* 76: 30-33.
- Wehrmann, V.K., Fehr, W.R., Cianzio, S.R. and Cavins, J.F. 1987. Transfer of High Seed Protein to High Yielding Soybean Cultivars. *Crop Sci.* 25: 927-931.
- Wesley, I.J., Osborne, B.G., Anderssen, R.S and Skerritt, J.H. 1999. Curve Fitting Applied to Near Infrared Deconvolution of Wheat Functional Proteins in Flour. *Near Infrared Spectroscopy, Proceedings of the International Conference, 9th, Verona, Italy*, p.215.
- Whistler, R. L. and BeMiller, J. N. 1997, *Carbohydrates Chemistry for Food Scientists*, Eagen Press, St. Paul, MN.
- Wilcox, J.R., Cavins, J.F., and Nielsen, N.C. 1984. Genetic Alteration of Soybean Oil Composition by a Chemical Mutagen. *Journal of the American Oil Chemists Society*, 61: 97-100.
- Wilcox, J.R., Cavins, J.F. 1995. Backcrossing High Seed Protein to a Soybean Cultivar. *Crop Science.* 35: 1036-1041.
- Wilcox, J.R. 1998. Increasing Seed Protein in Soybean with Eight Cycles of Recurrent Selection. *Crop Sci.* 38, 1536-1540.
- Wilcox J.R. and Shibles, R.M. 2001. Interrelationship among Seed Quality Attributes in Soybean, *Crop Sci.* 41: 11-14.
- Williams, P.C. 1975. Application of NIRS to Analysis of Cereal and Oilseeds. *Cereal Chem.* 52:561-576.
- Williams, P.C., Sobering, D.C. 1993. Comparison of Commercial Near Infrared Transmittance and Reflectance Instruments for Analysis of Whole Grains and Seeds. *J. Near Infrared Spectroscopy.* 1: 25-32.

- Williams, P.C., Norris, K. 1987. *Near-Infrared technology in the agricultural and food industries*. American Association of Cereal Chemists, Inc. St.Paul, MN. p330.
- Wilson, J. M., Kramer, A., and Ben-Gera, I. 1973. Quantitative Determination of Fat, Protein and Carbohydrates of Soy Products with Infrared Attenuated Total Reflectance. *J. Food Sci.* 38:14-17.
- Wilson, R. F. Advances in the Genetic Alteration of Soybean Oil Composition. 1991. In: R.F. Wilson (ed). *"Designing Value-Added Soybean for Markets of the Future."* American Oil Chemists Society, Champaign, Illinois p.38-53.
- Winkler, T., Bieschke, J. and Schwillie, P. 1997. Development of a Dual-Color Cross-Correlation System for FCS. www.mpibpc.gwdg.de/abteilungen/081/fcs/correlation/english.html.
- Winkler, T., Kettling, U., Koltermann, A., and Eigen, M. 1999. Confocal Fluorescence Coincidence Analysis: An Approach to Ultra High-Throughput Screening. *Proc. Natl. Acad. Sci. USA*, 96: 1375-1378.
- You, T. PhD Thesis, 2005, University of Illinois, Urbana, Illinois.
- You, T., Guo J., Baianu, I.C., 1999. Soybean Composition Analysis by Near Infrared Spectroscopy. *Global Soy1999*, Chicago, Illinois(Abstract).
- You, T., Guo, J., Baianu, I.C. and Nelson, R.L. 2002A. Determination of Isoflavones Contents for Selected Soybean Lines by Fourier Transform Near Infrared Reflectance Spectroscopy. *Proceedings for the 9th Biennial Conference of the Cellular and Molecular Biology of the Soybean*, August 11-14, 2002, p505.
- You T., Guo, J., Baianu, I.C., Nelson, R.L. 2002B. Rapid Determination of Protein, Oil, Moisture, and Isoflavones Contents of Single Soybean Seeds by Fourier Transform Near Infrared Reflectance Spectroscopy. *Proceedings of the China and International Soy Conference and Exhibition 2002 (CISCE 2002)*, November 6-9, 2002, 414-415.

